

This book was taken from the Library on the date  
last stamped. A fine of 1 anna or six annas will  
be charged for each day the book is kept overtime.

---

--	--	--	--



# CONTENTS

## JANUARY, NO. 1

<i>Studies of the growth and blood composition of dairy calves fed remade skim milk after three days of age.</i> L. R. ARRINGTON AND P. M. REAVES	1
<i>The effect of penicillin upon the livability, glycolysis, and bacterial content of bovine semen.</i> J. O. ALMQUIST, W. T. S. THORP, AND C. B. KNOTT	11
<i>The effect of conditions of storage and the viscosity of sweetened condensed milk.</i> B. H. WEBB AND C. F. HUFNAGEL	21
<i>Determination of cheese lipase.</i> MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE	31
<i>Lipase activity during making and ripening of Cheddar cheese.</i> MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE	39
<i>Determination of cheese proteinase.</i> MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE	47
<i>Proteinase content of Cheddar cheese during making and ripening.</i> MERLIN H. PETERSON, MARVIN J. JOHNSON AND WALTER V. PRICE	55
<i>The heritability of official type ratings and the correlation between type ratings and butterfat production of Ayrshire cows.</i> W. J. TYLER AND GEORGE HYATT, JR.	63
<i>Variations in type ratings of individual Ayrshire cows.</i> GEORGE HYATT, JR., AND W. J. TYLER	71
<i>The utilization of lactose by the dairy calf fed normal or modified milk diets.</i> JORGE ROJAS, B. S. SCHWEIGERT, AND I. W. RUPEL	81
<i>Association announcement</i>	89
<i>Abstracts of literature</i>	A1

## FEBRUARY, NO. 2

<i>The relationship of mammary development and body weight.</i> J. R. SCHABINGER AND C. B. KNOTT	95
<i>Permanency of synthetic ascorbic acid added to milk.</i> ARTHUR D. HOLMES AND CARLETON P. JONES	99
<i>Sulfamethazine blood and milk concentrations in dairy cows.</i> PETER H. LANGER, ROBERT L. BURKHART, CHARLES R. SCHROEDER, AND MARK WELSH	103
<i>The utilization of <math>\beta</math>-carotene, vitamin A alcohol, and the natural ester of vitamin A by Holstein heifers.</i> R. H. ROSS, C. B. KNOTT, AND N. B. GUERREANT	111
<i>Liveweight and milk-energy yield at various feeding intensities.</i> S. D. MUSGRAVE AND W. L. GAINES	119
<i>The chemical composition of the crystalline deposit in evaporated milk.</i> EDGAR F. DEYSHER AND B. H. WEBB	123
<i>The isolation and properties of the immune proteins of bovine milk and colostrum and their role in immunity: A review.</i> EMIL L. SMITH	127
<i>The utilization of whey: A review.</i> BYRON H. WEBB AND EARLE O. WHITTIER	139
<i>Abstracts of literature</i>	A17

## MARCH, NO. 3

<i>Effect of season, breed and species of ruminants on the vitamin A potency of butterfat.</i> B. C. RAY SARKAR	165
<i>Parturient paresis. II. The effect of partial versus complete milking upon the total blood serum calcium of dairy cows at parturition.</i> YEHL R. SMITH, R. P. NIEDERMEIER, AND R. G. HANSEN	173
<i>The value of hypochlorite and quaternary ammonium compounds, when used in udder washes, in reducing the plate count of milk.</i> E. M. KESLER, G. H. WATROUS, JR., C. B. KNOTT, AND P. S. WILLIAMS	179



<i>The nutrition of the newborn dairy calf. I. Changes in the tryptophan content of the blood plasma following birth and the ingestion of colostrum.</i> T. S. SUTTON AND G. C. ESH .....	183
<i>A comparison of vacuum and steam distillation for determining the volatile acidity of evaporated milk.</i> P. G. MILLER, P. L. ZIMMERMAN, AND E. B. OBERG .....	189
<i>Curd tension test and curd number test applied to market homogenized milk in Philadelphia—definition of a soft curd milk.</i> BERNHARD SPUR .....	199
<i>Variations in yield of milk under the penkeeping system in Brazil.</i> GERALDO G. CARNEIRO AND JAY L. LUSH .....	203
<i>Abstracts of literature</i> .....	A27

## APRIL, NO. 4

<i>A comparative study of the biochemical activity of Streptococcus lactis, Streptococcus citrovorus, and Streptococcus paracitrovorus when grown in cow's milk and soybean milk.</i> CHARLES W. GEHRKE AND HARRY H. WEISER .....	213
<i>The role of surface-active constituents involved in the foaming of milk and certain milk products. III. Milk lipids, including phospholipids.</i> G. A. RICHARDSON AND M. S. ELRAFEY .....	223
<i>A study of multiple births in a Holstein-Friesian herd.</i> K. O. PFAU, J. W. BARTLETT, AND C. E. SHUART .....	241
<i>A study of the browning reaction in whole milk powder and ice cream mix powder.</i> N. P. TARASSUK AND E. L. JACK .....	255
<i>Iron and copper content of non-milk products commonly used in ice cream.</i> HARRY PYENSON AND P. H. TRACY .....	269
<i>The development of flavor in American Cheddar cheese made from pasteurized milk with Streptococcus faecalis starter.</i> A. C. DAHLBERG AND F. V. KOSIKOWSKY .....	275
<i>The growth and survival of Streptococcus faecalis in pasteurized milk American Cheddar cheese.</i> F. V. KOSIKOWSKY AND A. C. DAHLBERG .....	285
<i>The tyramine content of cheese.</i> F. V. KOSIKOWSKY AND A. C. DAHLBERG .....	293
<i>The relationship of the amount of tyramine and the numbers of Streptococcus faecalis to the intensity of flavor in American Cheddar cheese.</i> A. C. DAHLBERG AND F. V. KOSIKOWSKY .....	305
<i>Determination of vitamin A in milk.</i> MELVIN HOCHBERG .....	315
<i>Abstracts of literature</i> .....	A43

## MAY, NO. 5

<i>The effect of clipping the udders of cows on the quality of milk.</i> E. W. HIRD, TULY REITER, K. G. WECKEL, AND N. N. ALLEN .....	325
<i>Storage and treatment of milking machine inflations under farm conditions.</i> J. M. JENSEN AND A. L. BORTREE .....	333
<i>Sweet potato meal versus ground corn in the ration of dairy cows.</i> JENNINGS B. FRYE, JR., JOHN H. THOMASON, AND HERBERT B. HENDERSON .....	343
<i>Correlation between the lactose content of milk and the cerebroside and choline content of brain.</i> D. P. SADHU .....	349
<i>Estimating the amount of feed derived from pasture by cows in the Connecticut Dairy Herd Improvement Association.</i> G. E. FRICK AND H. D. EATON .....	355
<i>Effect of water sprinkling with and without air movements on cooling dairy cows.</i> D. M. SEATH AND G. D. MILLER .....	363
<i>Measurement of fluorescent materials in milk and milk products.</i> ROBERT JENNESS AND S. T. COULTER .....	369
<i>The relationship of the change in pH effected by incubation to other semen characteristics.</i> J. T. REID, G. M. WARD, AND R. L. SALSBURY .....	385
<i>Association announcements</i> .....	391
<i>Abstracts of literature</i> .....	A61



# CONTENTS

V

## JUNE, NO. 6

<i>The spectrophotometric determination of the color of milk.</i> VICTOR NELSON .....	409
<i>The color of evaporated milk with respect to time and temperature of processing.</i> VICTOR NELSON .....	415
<i>Effect of raw soybeans and of soybean oil on plasma carotene and on vitamin A as measured by activated glycerol dichlorohydrin.</i> R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN .....	421
<i>Simple versus complex concentrate mixtures for young breeding bulls. I. Growth, blood composition, and cost.</i> J. T. REID, G. M. WARD, AND R. L. SALSBUURY .....	429
<i>Simple versus complex concentrate mixtures for young breeding bulls. II. Semen production.</i> J. T. REID, G. M. WARD, AND R. L. SALSBUURY .....	439
<i>A study of the use of the antioxidant nordihydroguaiaretic acid in dairy products. I. Its antioxygenic properties in milk.</i> J. W. STULL, E. O. HERREID, AND P. H. TRACY .....	449
<i>Vitamin D content of roughages.</i> J. A. NEWLANDER .....	455
<i>The effect of soya-phosphatides on the absorption and utilization of vitamin A in dairy animals.</i> G. C. ESH, T. S. SUTTON, J. W. HIBBS, AND W. E. KRAUSS .....	461
<i>Carotene and vitamin A in the colostrum of cows of typical Indian breeds.</i> B. C. RAY SARKAR .....	479
<i>Comparative antirachitic value of field-cured hay, barn-dried hay, and wilted grass silage for growing dairy calves.</i> L. A. MOORE, J. W. THOMAS, W. C. JACOBSON, C. G. MELIN, AND J. B. SHEPHERD .....	489
<i>The effect of streptomycin upon the livability and bacterial content of bovine semen.</i> J. O. ALMQUIST, P. J. GLANTZ, AND W. T. S. THORP .....	501
<i>Effect of ultraviolet irradiation on bacteriophage active against Streptococcus lactis.</i> G. I. GREENE AND F. J. BABEL .....	509
<i>Abstracts of literature</i> .....	A73

## JULY, NO. 7

<i>The keeping quality, solubility and density of powdered whole milk in relation to some variations in the manufacturing process. I. Keeping quality.</i> LOUIS J. MANUS AND U. S. ASHWORTH .....	517
<i>Beneficial effect and economic importance of using all colostrum produced in calf raising.</i> H. E. KAESER AND T. S. SUTTON .....	523
<i>Carotene requirements for Guernsey and Jersey calves as determined by spinal fluid pressure.</i> L. A. MOORE, J. F. SYKES, W. C. JACOBSON, AND H. G. WISEMAN .....	533
<i>Manufacture of powdered cream for whipping by aeration.</i> HARRY PYENSON AND P. H. TRACY .....	539
<i>Effect of delay in diluting and cooling on keeping quality of bull semen.</i> H. W. ANDERSON AND D. M. SEATH .....	551
<i>Effect of feeding tocopherols to dairy cows on the quantity and the fat content of milk produced.</i> T. W. GULLICKSON, J. B. FITCH AND L. O. GILMORE .....	557
<i>The elimination of interfering substances in the Kay-Graham phosphatase test when used for hard ripened cheese.</i> F. V. KOSIKOWSKY AND A. C. DAHLBERG .....	561
<i>Dehydrated sweet potatoes as a concentrate feed for dairy cattle.</i> ROBERT E. MATHER, WOODROW N. LINKOUS, AND JAMES F. EHEART .....	569
<i>A simple color test as an aid in grading farm-separated cream.</i> T. J. CLAYDON .....	577
<i>The effect of preparation of the cow on the rate of milking.</i> VEARL R. SMITH AND W. E. PETERSEN .....	589
<i>The reducing capacity of milk and milk products as measured by a modified ferricyanide method.</i> L. K. CROWE, ROBERT JENNESS, AND S. T. COULTER .....	595
<i>The influence of Mycotorula lipolytica lipase upon the ripening of blue cheese made from pasteurized homogenized milk.</i> I. I. PETERS AND F. E. NELSON .....	611



<i>Moisture studies in dry products of milk. II. Estimating water of crystallization of alpha-lactose in dry whey solids.</i> R. P. CHOI, C. M. O'MALLEY, AND B. W. FAIRBANKS .....	619
<i>Abstracts of literature</i> .....	A93

## AUGUST, NO. 8

<i>The nutritive value of homogenized milk: A review.</i> G. M. TROUT .....	627
<i>Abstracts of papers presented at the 43rd annual meeting</i> .....	657
<i>Proceedings of the 43rd annual meeting</i> .....	731
<i>Abstracts of literature</i> .....	A109

## SEPTEMBER, NO. 9

<i>The effect of pyridium, penicillin, furacin, and phenoxethol upon the livability of spermatozoa and upon the control of bacteria in diluted bull semen.</i> R. H. FOOTE AND G. W. SALISBURY .....	763
<i>The effect of sulfonamides upon the livability of spermatozoa and upon the control of bacteria in diluted bull semen.</i> R. H. FOOTE AND G. W. SALISBURY .....	769
<i>Breeding behaviour, spermatogenesis, and semen production of mature dairy bulls fed rations low in carotene.</i> R. W. BRATTON, G. W. SALISBURY, T. TANABE, C. BRANTON, E. MERCIER, AND J. K. LOOSLI .....	779
<i>Further studies of the nutritive value of butterfat fractions.</i> H. NATH, V. H. BARKI, C. A. ELVEHJEM, AND E. B. HART .....	793
<i>Sacral deformity in the "wrytail" abnormality in cattle.</i> LESTER O. GILMORE AND ALVIN F. SELLERS .....	797
<i>Frozen homogenized milk. IV. Keeping quality of frozen homogenized milk after thawing.</i> C. J. BABCOCK, CAPT. JOSEPH N. STABILE, ERNEST WINDHAM, LARUE B. EVANS, AND COL. RAYMOND RANDALL .....	805
<i>Frozen homogenized milk. V. Effect of age before freezing on the keeping quality of frozen homogenized milk.</i> C. J. BABCOCK, CAPT. JOSEPH N. STABILE, ERNEST WINDHAM, LARUE B. EVANS, AND COL. RAYMOND RANDALL .....	811
<i>Fertility level of bull semen diluted at 1:400 with and without sulfanilamide.</i> G. W. SALISBURY AND R. W. BRATTON .....	817
<i>An analysis of the results of the 1947 collegiate students' international contest in judging dairy products.</i> G. M. TROUT, E. O. ANDERSON, C. J. BABCOCK, P. A. DOWNS, AND F. H. HERZER .....	823
<i>Manometric measurement of the gas desorbed from vacuumized whole milk powder.</i> J. H. HETRICK AND P. H. TRACY .....	831
<i>Abstracts of literature</i> .....	A131

## OCTOBER, NO. 10

<i>The effect of agitation upon the livability of bovine spermatozoa.</i> P. W. PRINCE AND J. O. ALMQUIST .....	839
<i>Heat inactivation of milk phosphatase in dairy products.</i> GEORGE P. SANDERS AND OSCAR S. SAGER .....	845
<i>The adaptability of two strains of lactic streptococci to growth in the presence of homologous bacteriophage.</i> R. O. WAGENAAR AND C. C. PROUTY .....	859
<i>Effect of high-temperature short-time heat treatments on some properties of milk. I. Inactivation of the phosphatase enzyme.</i> J. H. HETRICK AND P. H. TRACY .....	867
<i>Effect of high-temperature short-time heat treatment on some properties of milk. II. Inactivation of the lipase enzyme.</i> J. H. HETRICK AND P. H. TRACY .....	881
<i>Properties of the colostrum of the dairy cow. II. Effect of prepartal rations upon the nitrogenous constituents.</i> D. B. PARRISE, G. H. WISE, J. S. HUGHES, AND F. W. ATKESON .....	889



## CONTENTS

vii

<i>The value of winter pasture and sweet potato meal for lactating dairy cows.</i> JENNINGS B. FRYE, JR., GEORGE E. HAWKINS, JR., AND HERBERT B. HENDERSON .....	897
<i>The determination of butterfat in ice cream employing mixed perchloric and acetic acids.</i> G. FREDERICK SMITH, J. S. FRITZ, AND HARRY PYENSON .....	905
<i>The nutrition of the newborn dairy calf. II. Effect of dietary tryptophan on the urinary excretion of niacin and its metabolites by young dairy calves.</i> G. C. ESH AND T. S. SUTTON .....	909
<i>The oxidized flavor in milk and dairy products: A review.</i> GEORGE R. GREENBANK .....	913
<i>Abstracts of literature</i> .....	A145

## NOVEMBER, NO. 11

<i>The keeping quality, solubility and density of powdered whole milk in relation to some variations in the manufacturing process. II. Solubility and density.</i> LOUIS J. MANUS AND U. S. ASHWORTH .....	935
<i>Effect of thyroxine on oxygen consumption of bovine spermatozoa and semen.</i> A. B. SCHULTZE AND H. P. DAVIS .....	946
<i>Retention of ascorbic acid, changes in oxidation-reduction potential, and the prevention of an oxidized flavor during freezing preservation of milk.</i> R. W. BELL .....	951
<i>Lipid deterioration in dairy products. The stability of milk fat and fat-soluble vitamins as determined by the re-emulsification test.</i> VLADIMIR N. KRUKOVSKY, E. S. GUTH- RIE, AND FRANK WHITING .....	961
<i>Lactating factors for dairy cows in dried grapefruit peel.</i> R. N. DAVIS AND A. R. KEMMERER .....	973
<i>The nutrition of the newborn dairy calf. III. The response to a photolyzed milk diet.</i> R. G. WARNER AND T. S. SUTTON .....	976
<i>Some changes in dry whole milk during storage.</i> S. T. COULTER, ROBERT JENNESS, AND L. K. CROWE .....	986
<i>Abstracts of literature</i> .....	A151

## DECEMBER, NO. 12

<i>In memory of Robert Bear Stoltz.</i> L. H. BURGWALD .....	1005
<i>The influence of water level and temperature of storage on carotene preservation in de- hydrated alfalfa, cereal grasses and mixed feeds.</i> A. W. HALVERSON AND E. B. HART .....	1008
<i>Studies on ruminal gas formation and on consumption of alfalfa pasture by cattle.</i> H. H. COLE AND MAX KLEIBER .....	1016
<i>A study of the use of the antioxidant nordihydroguaiaretic acid in dairy products. II. Its antioxygenic properties in unsweetened frozen cream.</i> J. W. STULL, E. O. HERREID AND P. H. TRACY .....	1024
<i>The relation between the month of calving and yearly butterfat production.</i> MOHAMED M. OLOUFA AND I. R. JONES .....	1029
<i>Some factors influencing the male hormone content of cow manure.</i> C. W. TURNER .....	1032
<i>The influence of the ration and rumen inoculation on the establishment of certain micro- organisms in the rumens of young calves.</i> J. W. HIBBS AND W. D. POUNDEN .....	1041
<i>The influence of the ratio of grain to hay in the ration of dairy calves on certain rumen microorganisms.</i> W. D. POUNDEN AND J. W. HIBBS .....	1051
<i>The influence of the ration and early rumen development on the changes in the plasma carotenoids, vitamin A, and ascorbic acid of young dairy calves.</i> J. W. HIBBS AND W. D. POUNDEN .....	1055
<i>The effect of supplemental vitamin A upon the growth, blood plasma carotene, vitamin A, inorganic calcium, and phosphorus of Holstein heifers.</i> R. H. ROSS AND C. B. KNODT .....	1062
<i>Collegiate students' international contest in judging dairy products</i> .....	1068

<i>Circulation of the Journal of Dairy Science</i> .....	1071
<i>Author index of original articles</i> .....	1108
<i>Subject index of original articles</i> .....	1117
<i>Abstracts of literature</i> .....	A159
<i>Author index of abstracts</i> .....	A167
<i>Subject index of abstracts</i> .....	A177
<i>Officers of the Association</i> .....	ii
<i>Table of contents</i> .....	viii
<i>Errata</i> .....	viii

## ERRATA, VOL. XXXI

- No. 4, page 297, lines 4 and 7. *Nitrate* should read *nitrite*.  
No. 8, page 717, line 32. *High* should read *low*.  
No. 9, page 768, line 18. *763-768* should read *769-777*.



# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

JANUARY, 1948

NUMBER 1

## STUDIES OF THE GROWTH AND BLOOD COMPOSITION OF DAIRY CALVES FED REMADE SKIM MILK AFTER THREE DAYS OF AGE<sup>1</sup>

L. R. ARRINGTON<sup>2</sup> AND P. M. REAVES

*Dairy Husbandry Department, Virginia Polytechnic Institute, Blacksburg*

The common method of feeding dairy calves for the first few weeks consists of feeding whole milk or partially skimmed milk for a limited time and then changing to skim milk. Other methods reported (3, 4, 10) involve changing abruptly to skim milk after the colostrum period and have proved satisfactory when the skim milk has been supplemented with vitamins A and D. Less success has been reported, however, with feeding remade skim milk. Morrow (11), in comparing fresh skim milk with remade skim milk, found that calves fed remade skim milk did not make as good growth as those fed fresh skim milk. Digestive disturbances also were noted in those calves fed the remade skim milk. Knott *et al.* (6) fed remade skim milk after 2 to 6 weeks of age and thereafter fed skim milk powder in the grain ration; they secured normal growth.

The partial success with feeding remade skim milk indicated the need for additional study of this method of feeding. The effects of this method would be expected to be exhibited, first, in the growth of the animals. The low-fat intake of the ration indicated a study of the blood fat content. Allen (2) has reported that the blood fat of dairy cattle is closely associated with feeding conditions. He reported that the blood of the newborn calf contains only a trace of fat, but the fat increases rapidly with the ingestion of food. This method of feeding also suggested a study of the blood carotene and vitamin A, since the skim milk must be supplemented with carotene or vitamin A.

### EXPERIMENTAL PROCEDURE

Seven Holstein and six Jersey male calves from the Virginia Polytechnic Institute dairy herd were used for this experiment. The seven Holstein calves and five of the Jersey calves were removed from their dams on the

Received for publication June 5, 1947.

<sup>1</sup>Data taken from thesis presented by L. R. Arrington as partial requirement for degree of Master of Science in Dairy Husbandry, 1941.

<sup>2</sup>Now at the University of Florida, College of Agriculture.

third day after birth and placed on the experimental ration. One of the Jersey calves was 10 days old when placed on the experiment and had received whole milk until that time. Each calf was housed in an individual stall throughout the experiment. An additional Holstein calf was used as a control, being handled in the same manner as the others except that fresh whole milk was fed in place of remade skim milk.

The experimental ration of remade skim milk was prepared by mixing 1 lb. of skim milk powder (spray process) with 9 lb. of warm water. This was fed at the rate of 1 lb. of milk daily per 10 lb. of body weight. A grain mixture was supplied as soon as the calves would eat it. The mixture consisted of 200 lb. of corn, 100 lb. of wheat, 250 lb. of whole oats, 250 lb. of wheat bran, 100 lb. of distillers grain, 100 lb. of linseed meal, 10 lb. of steamed bone meal, and 10 lb. of salt. Chopped red clover and alfalfa hay was supplied *ad libitum* as early as the calves would eat it.

One group of calves was fed a carotene concentrate;<sup>3</sup> the other group was fed cod-liver oil as the supplement. Those calves receiving the carotene supplement were given 20 drops of viosterol daily as a protection against vitamin D deficiency. The rate of carotene feeding was adjusted weekly with the increase in body weight. These concentrates were fed by mixing with the milk at the evening feeding.

Body weight and height at withers were taken at 3 days of age, when each calf was placed on the experiment, and each week thereafter, and blood fat determinations were made using the method described by Allen (1). Blood carotene determinations also were made weekly by the method of Moore (7), and blood vitamin A determinations were made weekly on six of the calves by the method of Kimble (5).

#### RESULTS

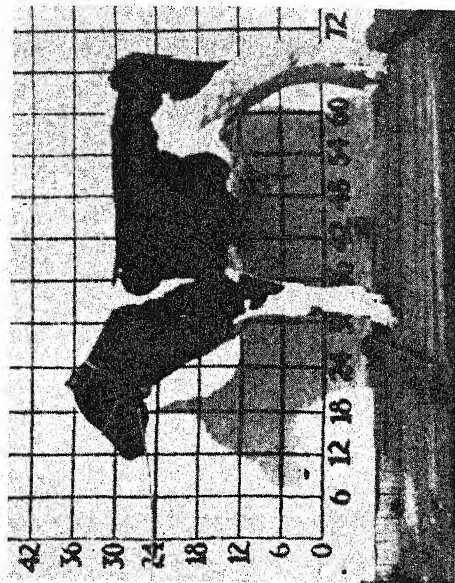
Six of the seven Holstein calves lived through the experimental period. Only one of the Jerseys (no. 812) lived through the experimental period, but this one was 10 days old when placed on experiment and had received whole milk until that time. Another Jersey calf (no. 813) lived until 14 weeks of age and died of bloating. Gain in body weight for this calf was slightly below normal. Since four of the six Jersey calves died within 2 to 4 weeks following the change to remade skim milk, they evidently could not withstand the abrupt change to the experimental ration or did not have the reserve to carry them over until they consumed feed other than milk. In each case where the calf died, scours occurred, and there was a steady loss in weight. Scours occurred in some of the other calves also, and it became necessary to reduce the milk intake. After the first 2 to 3 weeks, scours was much less prevalent, and animals appeared to be in better general health. There were no evidences of vitamin A or D deficiency.

<sup>3</sup> A commercial carotene preparation, Carotene—Type P-25, prepared and supplied by General Biochemicals, Inc.





Calf no. 819—Holstein fed carotene and viosterol. Age, 16 weeks; weight, 248 lb.



Calf no. 821—Holstein fed cod-liver oil. Age, 16 weeks; weight, 240 lb.

FIG. 1. Typical calves from each group receiving a supplement.

Table 1 shows the weekly weight of each calf throughout the experimental period. The Holsteins showed little or no gain for 2 or 3 weeks following the change to the experimental ration, and the Jerseys lost weight during this time. The retarded rate of gain is attributed to the low T.D.N. intake of the ration, since increases were nearly normal soon after grain and hay were consumed regularly. The intake of nutrients was below the requirement according to the Morrison Standard (9) until grain and hay were taken.

With the normal intake of grain and hay at about 6 weeks of age, increases in body weight were almost normal, and at 16 weeks of age the calves were practically normal size according to figures of Ragsdale (12).

Gains in height at withers were slightly below the normal rate (12) for the first few weeks. After about 3 weeks the Holsteins showed a normal increase in height, and after about 5 weeks the increase for the Jerseys compared favorably with the normal. There was no apparent difference in body weight and height at withers between the group fed carotene and the group fed cod-liver oil. Calves no. 819 and 821 were selected as typical calves from each group. Figure 1 shows a photograph of each.

Table 2 shows the weekly blood fat content of each calf. There were variations from week to week and there were differences among the animals, but there was no significant difference between the group fed carotene and the group fed cod-liver oil. Blood fat of the experimental calves was lower than that of the control calf fed whole milk. With the increase in intake of grain and hay, blood fat increased.

Blood carotene levels in those calves fed the carotene supplement were well above those reported as minimum requirements (8). The amount of blood carotene was considerably higher in this group than in the group fed cod-liver oil (table 3). There were slight variations from week to week in each group, but a general trend to a higher level toward the end of the experiment, when the calves were eating more hay, was apparent.

Blood vitamin A determinations were made on only six of the experimental animals, including the control. The plasma concentrations of those calves fed cod-liver oil as a supplement were higher than those of either the carotene group or the control calf (table 4). Concentrations for the control calf ranged intermediate between those of the cod-liver oil group and the carotene group.

#### SUMMARY

Seven Holstein and six Jersey male calves were fed an experimental ration of remade skim milk supplemented with vitamin A and D in the form of carotene and viosterol or cod-liver oil. The seven Holstein calves and five of the Jersey calves were placed on the experimental ration at 3 days of age. One of the Jersey calves received whole milk until 10 days of age. Grain and hay were fed as soon as the calves would eat it.



TABLE 1  
Body weight of experimental calves

No. of calf	Breed	3rd day	Age (weeks)																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
817*	H	75	75	77	84	90	103	113	125	140	145	155	179	193	207	219	237		
(lb.)																			
Carotene group																			
810	H	100	93	97	91	Died	62	70	76	85	90	103	109	119	132	145	167		
812	J	45	56	54	55	55													
814	J	45	47	45	Died														
815	H	92	93	99	108	112	124	125	135	149	163	168	183	197	211	224			
819	H	102	102	104	108	119	127	132	143	150	163	171	182	198	206	210	235	248	
820	H	97	97	98	103	108	112	120	127	136	147	157	164	177	186	192	209	228	
822	H	90	93	100	102	106	113	123	125	143	152	Experiment terminated							
823	J	47	45	44	43	41	Died												
824	J	51	50	47	41	Died													
Cod-liver oil group																			
813	J	55	52	50	51	55	62	64	74	82	94	96	105	115	128	Died of bloat			
816	J	50	44	43	Died														
818	H	84	82	80	85	85	91	99	113	120	133	142	153	164	179	185	194		
821	H	102	103	104	106	112	120	126	137	145	154	165	180	190	199	200	227	240	

\*Control calf.

TABLE 2  
Fat content of blood plasma

No. of calf	Breed	3rd day	Age (weeks)																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16					
			(mg./ml.)																				
817*	H	29	49	57	78		87	140	135									123	154	149	164	146	142
Carotene group																							
812	J				15	16	14	31	76	39	32	47	48	86	87	118	69						
815	H					24	24	23	35	55	46	83	70	52	29	50							
819	H		22	42					40	35	83	65	128	144	178	218	208	155					
820	H	40	27		11	11	23	38	24	44	44	93	99	158	189	190	132	133					
822	H		24	25	34	65	69	105	106	89	49	64	Experiment terminated										
823	J	47	110	78			Died																
824	J	20	63				Died																
Cod-liver oil group																							
813	J							53	27	20	38	22	53	39						Died (of bloat)			
816	J	24	20	10	Died																		
818	H	25	19	20	22	16	29	24	31	31	31	36	46	51	75	129	131						
821	H	50	26		32	23	12	15	26	27	54	110	87	157	180	173	126	119					

\*Control calf.



TABLE 3

*Carotene content of blood plasma*

No. of calf	Breed	3rd day	Age (weeks)																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16									
817*	H	0.26	0.16	0.15	0.08		0.11	0.21	0.08	(μg./ml.)										0.16	0.22	0.54	0.48	0.49	0.45	0.59	0.62
Carotene group																											
812	J						0.15	0.39	0.39	0.23	0.54	0.40	0.41	0.74	0.58	0.42	0.85	0.59									
815	H	0.34	0.10	0.20	0.11	0.24	0.25	0.25	0.45	0.26	0.11	0.48	0.45	0.31	0.58												
819	H		0.12	0.18		0.42	0.45	0.49	0.44	0.44	0.42	0.66	0.92	1.34	1.03	1.03	1.12	0.76									
820	H	0.12	0.11		0.30	0.18	0.38	0.35	0.35	0.35	0.50	0.58	0.84	0.72	0.70	0.80	0.49	0.47									
822	H		0.25	0.17	0.27	0.26	0.41	0.42	0.46	0.45	0.15	0.16	Experiment terminated														
Cod-liver oil group																											
813	J				0.10	0.20	0.20	0.25	0.15	0.30	0.28	0.14	0.34		0.21	0.21	Died (of bloat)										
818	H	0.05	0		0.09	0.03		0.04	0.04	0.05		0.15	0.16	0.30	0.47	0.61											
821	H	0.23	0.07		0.03		0.03	0.04	0.04	0.23	0.12	0.16	0.14	0.14	0.11	0.12	0.08	0.14									

\* Control calf.

TABLE 4  
*Blood vitamin A*

No. of Breed calf	Age (weeks)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
817* H									( $\mu\text{g./ml.}$ ) 0.08	0.10		0.07	0.13	0.18	0.13	0.10
Carotene group																
819 H			0.08	0.05		0.04	0.06	0.08	0.05			0.04	0.07	0.06	0.06	0.08
820 H			0.09	0.07		0.05	0.07	0.07	0.07	0.06	0.04	0.03	0.05	0.05	0.10	0.09
822 H	0.15	0.06	0.03		0.03	0.03	0.04	0.02	0.03	Experiment terminated						
Cod-liver oil group																
818 H							0.23	0.21		0.14	0.21	0.23	0.18	0.13	0.15	
821 H			0.16	0.10		0.17	0.17	0.12	0.11	0.10	0.11	0.16	0.17	0.15	0.19	0.18

\* Control calf.



Six of the seven Holstein calves lived through the experimental period of about 16 weeks. Growth rates were below normal for the first few weeks, but Holstein calves were practically normal size at 16 weeks of age. Four of the six Jersey calves died within 2 to 4 weeks following the change to the experimental ration. One of the other Jerseys died at 14 weeks of age from bloat, and only one Jersey lived through the 16-week experimental period. This one received whole milk until 10 days of age. There was no difference in growth rates between the groups fed the different vitamin supplements.

Blood fat content was lower in calves fed the experimental ration than that of the control calf fed whole milk. As the quantity of grain and hay consumed increased, the blood fat increased.

Blood carotene was higher in calves receiving the carotene supplement than that of the control calf on whole milk or that of the experimental calves receiving cod-liver oil as the supplement.

Blood vitamin A of calves fed cod-liver oil was higher than that of the control calf or that of the group receiving carotene as the supplement.

#### REFERENCES

- (1) ALLEN, N. N. Blood Fat of Dairy Cattle. I. A Simple Volumetric Method for Determining Blood Fat. Minn. Agr. Expt. Sta. Tech. Bull. 130, pp. 1-17. 1938.
- (2) ALLEN, N. N. Blood Fat of Dairy Cattle. II. Factors Influencing the Fat Content of the Blood Plasma. Minn. Agr. Expt. Sta. Tech. Bull. 130, pp. 17-52. 1938.
- (3) ARCHER, J., ARCHER, J. E., AND BOND, J. R. The Rearing of Calves from Birth without Whole Milk. Scot. J. Agr., 21: 259-267. 1938.
- (4) CONVERSE, H. T., AND MEIGS, E. B. Vitamin A Replaces Whole Milk in the Calf Ration. J. Dairy Sci., 19: 435-436. 1936.
- (5) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. J. Lab. Clin. Med., 24: 1055-1065. 1939.
- (6) KNOTT, J. C., HODGSON, R. E., AND ELLINGTON, E. V. Raising Dairy Calves with Dried Skim Milk. Wash. Agr. Expt. Sta. Bull. 273. 1932.
- (7) MOORE, L. A. The Determination of Blood Plasma Carotene in the Bovine Using a Photoelectric Colorimeter. J. Dairy Sci., 22: 501-511. 1939.
- (8) MOORE, L. A. Carotene Intake, Level of Blood Plasma Carotene and the Development of Papillary Edema and Nyctalopia in Calves. J. Dairy Sci., 22: 803-812. 1939.
- (9) MORRISON, F. B. Feeds and Feeding. 20th Ed. The Morrison Publishing Co., Ithaca, N. Y. 1946.
- (10) MORROW, K. S. Cod Liver Oil Helps Calves. N. H. Agr. Expt. Sta. Bull. 304, pp. 9-10. 1938.
- (11) MORROW, K. S. Dry-feed Systems of Raising Dairy Calves. N. H. Agr. Expt. Sta. Bull. 319, pp. 31-33. 1940.
- (12) RAGSDALE, A. C. Growth Standards for Dairy Cattle. Mo. Agr. Expt. Sta. Bull. 336. 1934.





## THE EFFECT OF PENICILLIN UPON THE LIVABILITY, GLYCOLYSIS, AND BACTERIAL CONTENT OF BOVINE SEMEN<sup>1,2</sup>

J. O. ALMQUIST,<sup>3</sup> W. T. S. THORP,<sup>4</sup> AND C. B. KNOTT<sup>3</sup>

*Pennsylvania Agricultural Experiment Station, State College*

One of the most important problems in the artificial breeding of dairy cattle at the present time is the control of the bacterial flora of diluted bull semen. While present-day techniques in the collection of bull semen under routine artificial breeding conditions tend to reduce bacterial contamination, it is still impossible to obtain semen which is absolutely free of bacteria. Unless adequate precautions are taken in the preparation and handling of the diluted semen, further contamination may occur. In addition, both undiluted and diluted semen serve as excellent media for bacterial growth.

It is believed that the semen of the bull may serve as a means of transmitting certain genital infections which are related to fertility problems in a dairy herd. The widespread use of artificial breeding has greatly magnified the seriousness of this problem.

On the basis of the foregoing facts, the addition to semen of an anti-bacterial substance which does not exert an injurious effect on the spermatozoa would be of value in artificial breeding under field conditions.

As early as 1917, Ivanov (3) recognized and investigated the possibility of controlling the spread of infection by adding certain chemical substances, namely, ethyl alcohol, atoxyl, and salvarsan, to contaminated semen. In 1940 Shettles (8) reported that the survival and activity of human spermatozoa were not reduced by sulfanilamide or sulfapyridine in concentrations as high as 160 mg. per 100 ml. of the Baker's fluid used for diluting purposes. However, Knodt and Salisbury (4) were the first investigators to study the feasibility of using certain bacteriostatic compounds to control bacterial growth in bull semen. Using sulfanilamide at levels ranging from 0 to 1,000 mg. per 100 ml. of yolk-citrate diluter, they found that levels of 200 mg. and over controlled the growth of bacteria in stored diluted semen (20 days' storage at 5° C.). They concluded that 300 mg. of sulfa-

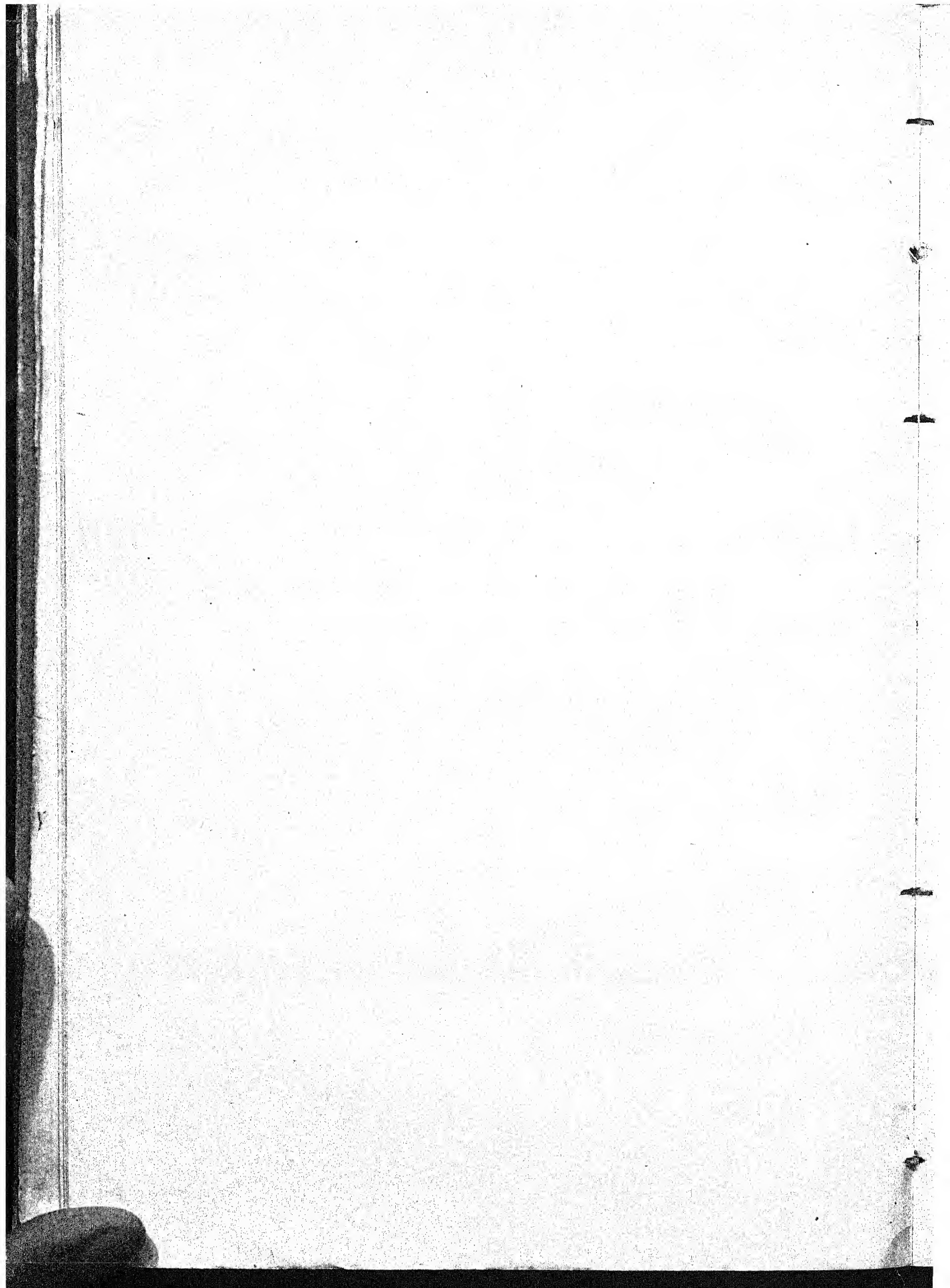
Received for publication September 17, 1947.

<sup>1</sup> Authorized for publication September 12, 1947, as paper no. 1390 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This study was supported in part by funds granted by Charles Pfizer and Co., Inc., Brooklyn, New York, who also provided the penicillin.

<sup>2</sup> The data contained in this paper are part of a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

<sup>3</sup> Department of Dairy Husbandry.

<sup>4</sup> Now with the National Institute of Health, Bethesda, Maryland.



## THE EFFECT OF PENICILLIN UPON THE LIVABILITY, GLYCOLYSIS, AND BACTERIAL CONTENT OF BOVINE SEMEN<sup>1,2</sup>

J. O. ALMQUIST,<sup>3</sup> W. T. S. THORP,<sup>4</sup> AND C. B. KNOTT<sup>3</sup>

*Pennsylvania Agricultural Experiment Station, State College*

One of the most important problems in the artificial breeding of dairy cattle at the present time is the control of the bacterial flora of diluted bull semen. While present-day techniques in the collection of bull semen under routine artificial breeding conditions tend to reduce bacterial contamination, it is still impossible to obtain semen which is absolutely free of bacteria. Unless adequate precautions are taken in the preparation and handling of the diluted semen, further contamination may occur. In addition, both undiluted and diluted semen serve as excellent media for bacterial growth.

It is believed that the semen of the bull may serve as a means of transmitting certain genital infections which are related to fertility problems in a dairy herd. The widespread use of artificial breeding has greatly magnified the seriousness of this problem.

On the basis of the foregoing facts, the addition to semen of an antibacterial substance which does not exert an injurious effect on the spermatozoa would be of value in artificial breeding under field conditions.

As early as 1917, Ivanov (3) recognized and investigated the possibility of controlling the spread of infection by adding certain chemical substances, namely, ethyl alcohol, atoxyl, and salvarsan, to contaminated semen. In 1940 Shettles (8) reported that the survival and activity of human spermatozoa were not reduced by sulfanilamide or sulfapyridine in concentrations as high as 160 mg. per 100 ml. of the Baker's fluid used for diluting purposes. However, Knodt and Salisbury (4) were the first investigators to study the feasibility of using certain bacteriostatic compounds to control bacterial growth in bull semen. Using sulfanilamide at levels ranging from 0 to 1,000 mg. per 100 ml. of yolk-citrate diluter, they found that levels of 200 mg. and over controlled the growth of bacteria in stored diluted semen (20 days' storage at 5° C.). They concluded that 300 mg. of sulfa-

Received for publication September 17, 1947.

<sup>1</sup> Authorized for publication September 12, 1947, as paper no. 1390 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This study was supported in part by funds granted by Charles Pfizer and Co., Inc., Brooklyn, New York, who also provided the penicillin.

<sup>2</sup> The data contained in this paper are part of a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

<sup>3</sup> Department of Dairy Husbandry.

<sup>4</sup> Now with the National Institute of Health, Bethesda, Maryland.



nilamide per 100 ml. of diluter was the most favorable level since it not only prevented bacterial growth but also brought about a significant increase in spermatozoan livability. Further studies by these workers (6) more recently have shown that the addition of sulfanilamide to diluter at this optimal level gave an increase in the fertility of bull semen used in the routine artificial breeding of dairy cows. They attributed the beneficial effects of sulfanilamide on fertility to changes in the metabolism of the spermatozoa rather than to the control of bacterial growth alone.

Recently the possibility of using other antibacterial agents to prevent bacterial growth in bull semen has been reported. In attempts to produce a synthetic diluting medium which would be superior to the egg yolk-buffer diluters commonly used at the present time, Phillips and Spitzer (5) noticed considerable growth of bacteria in some of the preparations. After studying the effect of various antibacterial substances upon the motility of spermatozoa during storage, they recommended that 0.03 per cent of sulfathalidine, sulfasuxidine, or streptomycin be added to their newly developed lipid-glucose-buffer-gum (LGB) diluter to control bacterial contamination. They also mentioned that penicillin was not deleterious to spermatozoan motility but did not recommend its use in their LGB diluter.

Since penicillin is recognized as one of the most effective antibiotic agents in treating a variety of infections and is unique because of low tissue toxicity, it seemed desirable to study its use in bovine semen. Thus laboratory and field experiments were undertaken to determine the effects of penicillin upon the bacterial content of diluted bovine semen and its influence on the livability, metabolism, and fertility of the spermatozoa. The effect of penicillin upon fertility still is under investigation and will be reported later.

#### EXPERIMENTAL

*Effect of penicillin upon spermatozoan livability.* Preliminary studies to determine the effect of penicillin upon spermatozoan livability indicated that levels of 2,500 up to 10,000 Oxford units of penicillin per ml. of diluted semen were definitely detrimental. Thus, penicillin was added to 12 samples of bull semen at the rate of 0, 250, 500, 750, 1,000, 1,250, 1,500, and 2,000 Oxford units per ml. of diluted semen. The 12 ejaculates were diluted at the constant rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. This dilution was selected because it represented the average dilution rate being used by the artificial breeding cooperatives in Pennsylvania at the time the experiment was designed. The yolk-citrate diluter was composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g. of  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  in 100 ml. of water distilled over glass. When the design of the experiment involved the addition of penicillin, the desired amount of this substance was dissolved in the sodium citrate solution and mixed with the egg yolk in order to ensure the preparation of a diluter which would have a 1:1 ratio of yolk to buffer.

To test the effect of penicillin upon spermatozoan livability, the diluted semen was stored at 4.5°C. and the percentages of actively motile spermatozoa were determined every 2 days for a period of 20 days. At 0, 8, and 16 days of storage, subsamples were taken for glucose and lactic acid determinations, as well as for bacterial counts and penicillin assays.

The 12 ejaculates studied had a mean concentration of 1,147,000 spermatozoa per cubic millimeter, a mean motility of 70 per cent active spermatozoa, and a mean methylene blue reduction time of 7.2 minutes (range 5.0 to 10.0 minutes).

The results of the motility observations are shown in table 1. Each figure represents a mean of 12 ejaculates. Analysis of variance involving a total of 960 motility estimations showed no significant differences in livability between the 0, 250, 500, and 750 unit levels of penicillin. The 1,000,

TABLE 1  
*The effect of penicillin upon the livability of spermatozoa*

Penicillin units per ml. diluted semen	Per cent motile spermatozoa (12 ejaculates)					
	Before storage	After storage at 4.5° C. for				
		4 days	8 days	12 days	16 days	20 days
Control	70	52	41	31	17	11
250	70	52	40	30	16	11
500	70	52	40	27	17	7
750	70	54	40	27	16	7
1000	70	52	39	26	16	6
1250	70	51	37	25	14	4
1500	70	47	33	22	12	4
2000	70	45	32	21	10	2

1,250, 1,500, and 2,000 unit levels of penicillin brought about a highly significant ( $P = <0.01$ ) decline in the ability of the spermatozoa to maintain motility as compared to the untreated controls. Highly significant differences between ejaculates and between storage intervals were found, as well as highly significant ejaculate  $\times$  treatment and storage interval  $\times$  ejaculate interactions. Apparently the various ejaculates responded differently to the several levels of penicillin in their livability during storage.

An examination of the mean motility observations for each treatment indicated that there was a more or less uniform decline in livability with increased amounts of penicillin. Thus, the relationship between spermatozoan livability and level of penicillin also was studied by means of regression. Both highly significant linear and curvilinear regressions were obtained (fig. 1). However, a test of significance of departure from linearity showed that the linear regression represented the livability data more accurately. Based on linear regression, the mean percentage of motile spermatozoa during 20 days' storage decreased by 0.9 per cent for each additional 250 units of penicillin as compared to the untreated controls.

Since in routine artificial breeding practically all semen is used by the time it is 6 days of age, an analysis of variance was made which included only the motility data for 2, 4, and 6 days' storage. According to the least differences required for significance, levels of penicillin up to and including 1,000 units did not cause a mathematically significant decrease in livability as compared to the untreated controls, but the levels of 1,250, 1,500, and 2,000 units brought about a highly significant decline in spermatozoan activity.

*Effect of penicillin upon the glycolysis of spermatozoa.* The studies of Knodt and Salisbury (4) indicated that the percentage recovery of glucose

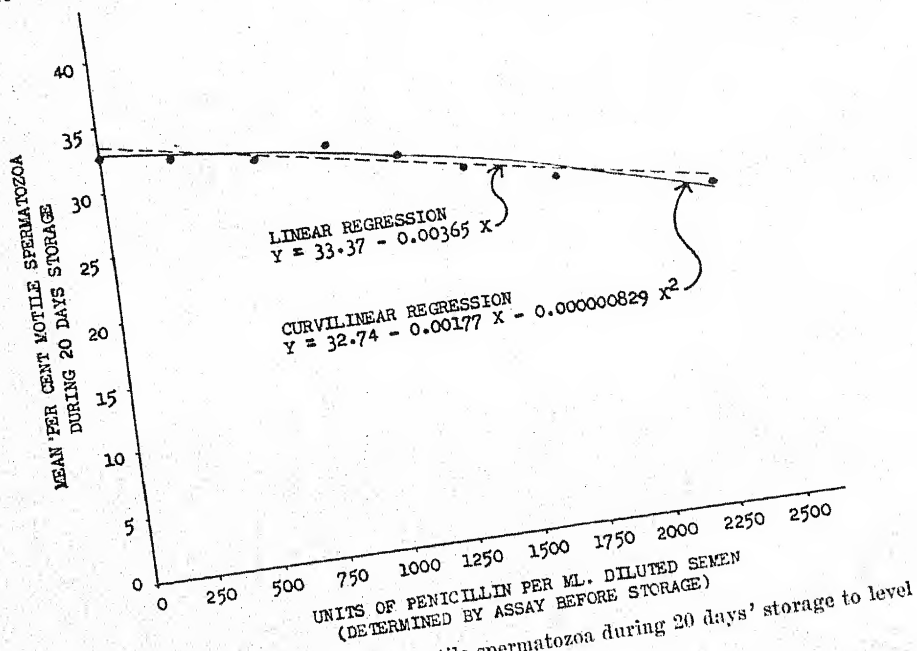


FIG. 1. Relationship of per cent motile spermatozoa during 20 days' storage to level of penicillin, as shown by regression.

as lactic acid was relatively small in diluted bull semen containing no sulfanilamide, but increased when the sulfanilamide was added. In the present study, glucose and lactic acid determinations were made on nine samples of diluted semen stored for 0, 8, and 16 days. Glucose analyses were made according to the method of Somogyi (9), while lactic acid was measured by the method of Barker and Summerson (2). The data are shown in table 2. Analysis of variance involving 216 glucose determinations demonstrated that penicillin significantly reduced the utilization of glucose at all levels. On the other hand, an analysis of the corresponding lactic acid determinations showed no statistically significant differences between the controls and



the various levels of penicillin. The addition of penicillin increased the percentage of glucose loss recovered as lactic acid as compared to the tubes without penicillin. Thus, bacteria may have utilized part of the glucose not recovered as lactic acid in the diluted semen which was not treated with penicillin.

*Effect of penicillin upon the bacterial content of diluted semen.* Bacterial plate counts were made on five samples of diluted semen using veal infusion agar containing 2 per cent sterile defibrinated ox blood. The samples were plated at dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 with incubation at 37° C. for 72 to 96 hours. Organisms of the coliform group were determined by counts on desoxycholate agar plates which were examined after 24 hours at 37° C. and, if no colonies were present, after an additional 24 to 48 hours. Bacterial counts were made not only on diluted semen after 0, 8, and 16 days of storage, but also on samples of undiluted semen and yolk-citrate diluter stored for the same intervals.

TABLE 2

*The effect of penicillin upon the glycolysis of diluted bull semen stored for 16 days at 4.5° C. (mean of 9 determinations)*

	Units of penicillin per ml. of diluted semen							
	0	250	500	750	1000	1250	1500	2000
Mg. % glucose* loss ...	63	46	58	51	43	52	49	44
Mg. % lactic acid gain .....	46	47	47	49	48	49	46	42
% glucose utilized recovered as lactic acid .....	73	102	81	96	112	94	94	95

\* Reducing substances expressed as glucose.

In the five semen samples studied, no growth of typical coliform colonies on the desoxycholate agar plates was noted.

The data presented in table 3 indicate that penicillin retarded bacterial growth in freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was observed in the tubes without penicillin. The majority of the plates made from both penicillin-treated and untreated semen stored for 16 days possessed a heavy growth of minute colonies which made counting impossible. In four out of 20 possible cases, countable plates were obtained at the 250 and 500 unit levels from the tubes stored for 0 and 8 days. Possibly the raw semen contained bacteria which were relatively resistant to penicillin at these lower concentrations but which were inhibited by the higher concentrations.

The three irregular plate counts obtained in sample numbers 13 and 14 at levels of 1,000, 1,500, and 2,000 units (after storage for 8 days), as well as the heavy growth of pin-point colonies noted at 16 days (table 3), may

TABLE 3  
The effect of penicillin upon bacterial growth in diluted semen stored for 8 and 16 days at 4.5° C. (5 ejaculates)

Bull	Sample no.	Bacteria per ml. (in thousands)									
		Undiluted semen	Yolk-citrate diluter	Units of penicillin per ml. of diluted semen							
				0	250	500	750	1000	1250	1500	2000
Before storage											
B	11	0.2	*	*	*	*	*	*	*	*	*
C	12	7.7	*	0.6	*	*	*	*	*	*	*
B	13	1.6	*	0.7	*	*	*	*	*	*	*
A	14	960	*	76	41	*	*	*	*	*	*
B	15	850	*	50	54	25	*	*	*	*	*
After storage for 8 days											
B	11	0.5	*	*	*	*	*	*	*	*	*
C	12	4.5	*	225	*	*	*	*	*	*	*
B	13	0.6	*	1.7	*	*	*	*	*	*	*
A	14	200	0.3	75	39	*	*	40	*	*	*
B	15	340	*	15	*	*	*	*	*	6.8	0.8
After storage for 16 days											
B	11	0.6	78	†	†	†	†	†	†	†	†
C	12	3	*	10	*	*	*	*	*	*	*
B	13	0.5	†	†	†	†	†	†	†	†	†
A	14	1600	*	†	†	†	†	†	†	†	†
B	15	2500	*	15	†	†	†	†	†	†	†

\* = < 300 bacteria per ml.  
† = > 3,000,000 bacteria per ml.

\* = < 300 bacteria per ml.

† = > 3,000,000 bacteria per ml. (pin-point colonies too numerous to count).

be due to bacterial contamination of the samples during storage. That is, the tubes from which the subsamples for plate counts were taken were opened every 2 days during storage in order to make motility observations on the spermatozoa. Thus, it is possible that penicillin-resistant organisms were added to the samples. The bacterial counts on the yolk-citrate diluter stored for 8 days were the same, with only one exception, as those obtained prior to storage. These tubes were not opened during the 8-day storage period and thus contamination apparently was avoided.

Total plate counts on portions of freshly collected semen showed considerable variation, ranging from 200 to 960,000 bacteria per ml. with a mean of 364,000 bacteria per ml. for the five ejaculates studied. The semen samples were collected by means of an artificial vagina from three bulls used

TABLE 4

*The stability of penicillin in diluted semen stored at 4.5° C. (mean of 9 determinations)*

Theoretical units of penicillin*	Units of penicillin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
250	266	270	233
500	592	568	468
750	857	815	719
1000	1133	1152	963
1250	1370	1313	1109
1500	1707	1718	1393
2000	2318	2156	1952
Diluter alone	0	0	0

\* Number of units expected based on the total units in the ampules according to the producer.

for natural breeding in the College herd and no special precautions were taken to clean the bulls prior to collection.

*Stability of penicillin in diluted semen.* Penicillin assays were made on nine samples of diluted semen after storage for 0, 8, and 16 days, not only to determine the stability of the antibiotic but also to obtain more definite information on the actual number of units of penicillin added to the tubes of diluted semen. The latter was of particular interest since producers of penicillin usually add some excess units of penicillin to their ampules. Assays were made according to the standard cylinder plate method of Schmidt and Moyer (7). *Staphylococcus aureus* was the test organism employed. Plain yolk-citrate diluter was also assayed against this organism in order to ascertain whether or not it had any antibacterial activity.

The data in table 4 show that there was practically no loss of penicillin activity in diluted semen stored for 8 days at 4.5° C. Even after storage



for 16 days only a slight decrease in concentration occurred. The temperature employed, as well as the fact that apparently no coliform organisms were present in the samples, was undoubtedly responsible to a large extent for the results obtained. Organisms of the coliform group, particularly *Escherichia coli* (1), are capable of producing a penicillinase which destroys penicillin.

As indicated in table 4 the units of penicillin obtained by assay on portions of the diluted semen immediately following preparation always exceeded the theoretical number of units. Assays on yolk-citrate diluter showed that it possessed no antibiotic activity.

#### SUMMARY

1. The addition of 250, 500, and 750 Oxford units of penicillin per ml. of diluted semen did not significantly reduce the ability of spermatozoa to maintain motility during a storage period of 20 days. Levels of penicillin ranging from 1,000 to 2,000 units per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a 20-day storage period.

2. In routine artificial breeding, semen is seldom used after holding more than 6 days. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6-day storage period occurred as the result of addition of 250, 500, 750, or 1,000 units of penicillin per ml. of diluted semen, but higher levels of penicillin were deleterious.

3. Within the limits of laboratory experiment, the relation between spermatozoan livability and level of penicillin is well represented by a straight line, the mean percentage of motile spermatozoa during storage for 20 days decreasing by 0.9 per cent for each additional 250 units of penicillin.

4. Penicillin depressed the utilization of glucose at all levels studied (250 to 2,000 units per ml. of diluted semen), while the amounts of lactic acid which accumulated were not significantly affected. Addition of penicillin increased the percentage of glucose utilized which was recovered as lactic acid.

5. Penicillin retarded bacterial growth at all levels in both freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was found in the semen without penicillin. The initial plate counts for the five ejaculates studied ranged from 200 to 960,000 bacteria per ml. of undiluted semen, with a mean of 364,000 bacteria per ml.

6. There was no appreciable loss in penicillin activity in diluted semen stored for 8 days and only a slight decrease in concentration after storage for 16 days at 4.5° C.

## REFERENCES

- (1) ABRAHAM, E. P., AND CHAIN, E. An Enzyme from Bacteria Able to Destroy Penicillin. *Nature*, 146: 837. 1940.
- (2) BARKER, S. B., AND SUMMERSON, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. *J. Biol. Chem.*, 138: 535-554. 1941.
- (3) IVANOV, E. Method of Rendering Infected Mammalian Sperm Incapable of Transmitting Infection. *Compt. rend. soc. biol.*, 80: 765-767. 1917. (*Chem. Abs.*, 12: 47-48. 1918).
- (4) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (5) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (6) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (7) SCHMIDT, W. H., AND MOYER, A. J. Penicillin. I. Methods of Assay. *J. Bact.*, 47: 199-209. 1944.
- (8) SHETTLES, L. B. Resistance of Human Spermatozoa *in vitro* to Sulfanilamide and Sulfapyridine. *Proc. Soc. Exptl. Biol. Med.*, 44: 392-395. 1940.
- (9) SOMOGYI, M. Determination of Blood Sugar. *J. Biol. Chem.*, 160: 69-73. 1945.

for 16 days only a slight decrease in concentration occurred. The temperature employed, as well as the fact that apparently no coliform organisms were present in the samples, was undoubtedly responsible to a large extent for the results obtained. Organisms of the coliform group, particularly *Escherichia coli* (1), are capable of producing a penicillinase which destroys penicillin.

As indicated in table 4 the units of penicillin obtained by assay on portions of the diluted semen immediately following preparation always exceeded the theoretical number of units. Assays on yolk-citrate diluter showed that it possessed no antibiotic activity.

#### SUMMARY

1. The addition of 250, 500, and 750 Oxford units of penicillin per ml. of diluted semen did not significantly reduce the ability of spermatozoa to maintain motility during a storage period of 20 days. Levels of penicillin ranging from 1,000 to 2,000 units per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a 20-day storage period.

2. In routine artificial breeding, semen is seldom used after holding more than 6 days. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6-day storage period occurred as the result of addition of 250, 500, 750, or 1,000 units of penicillin per ml. of diluted semen, but higher levels of penicillin were deleterious.

3. Within the limits of laboratory experiment, the relation between spermatozoan livability and level of penicillin is well represented by a straight line, the mean percentage of motile spermatozoa during storage for 20 days decreasing by 0.9 per cent for each additional 250 units of penicillin.

4. Penicillin depressed the utilization of glucose at all levels studied (250 to 2,000 units per ml. of diluted semen), while the amounts of lactic acid which accumulated were not significantly affected. Addition of penicillin increased the percentage of glucose utilized which was recovered as lactic acid.

5. Penicillin retarded bacterial growth at all levels in both freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was found in the semen without penicillin. The initial plate counts for the five ejaculates studied ranged from 200 to 960,000 bacteria per ml. of undiluted semen, with a mean of 364,000 bacteria per ml.

6. There was no appreciable loss in penicillin activity in diluted semen stored for 8 days and only a slight decrease in concentration after storage for 16 days at 4.5° C.



## REFERENCES

- (1) ABRAHAM, E. P., AND CHAIN, E. An Enzyme from Bacteria Able to Destroy Penicillin. *Nature*, 146: 837. 1940.
- (2) BARKER, S. B., AND SUMMERSON, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. *J. Biol. Chem.*, 138: 535-554. 1941.
- (3) IVANOV, E. Method of Rendering Infected Mammalian Sperm Incapable of Transmitting Infection. *Compt. rend. soc. biol.*, 80: 765-767. 1917. (*Chem. Abs.*, 12: 47-48. 1918).
- (4) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (5) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (6) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (7) SCHMIDT, W. H., AND MOYER, A. J. Penicillin. I. Methods of Assay. *J. Bact.*, 47: 199-209. 1944.
- (8) SHETTLES, L. B. Resistance of Human Spermatozoa *in vitro* to Sulfanilamide and Sulfapyridine. *Proc. Soc. Exptl. Biol. Med.*, 44: 392-395. 1940.
- (9) SOMOGYI, M. Determination of Blood Sugar. *J. Biol. Chem.*, 160: 69-73. 1945.

for 16 days only a slight decrease in concentration occurred. The temperature employed, as well as the fact that apparently no coliform organisms were present in the samples, was undoubtedly responsible to a large extent for the results obtained. Organisms of the coliform group, particularly *Escherichia coli* (1), are capable of producing a penicillinase which destroys penicillin.

As indicated in table 4 the units of penicillin obtained by assay on portions of the diluted semen immediately following preparation always exceeded the theoretical number of units. Assays on yolk-citrate diluter showed that it possessed no antibiotic activity.

#### SUMMARY

1. The addition of 250, 500, and 750 Oxford units of penicillin per ml. of diluted semen did not significantly reduce the ability of spermatozoa to maintain motility during a storage period of 20 days. Levels of penicillin ranging from 1,000 to 2,000 units per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a 20-day storage period.

2. In routine artificial breeding, semen is seldom used after holding more than 6 days. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6-day storage period occurred as the result of addition of 250, 500, 750, or 1,000 units of penicillin per ml. of diluted semen, but higher levels of penicillin were deleterious.

3. Within the limits of laboratory experiment, the relation between spermatozoan livability and level of penicillin is well represented by a straight line, the mean percentage of motile spermatozoa during storage for 20 days decreasing by 0.9 per cent for each additional 250 units of penicillin.

4. Penicillin depressed the utilization of glucose at all levels studied (250 to 2,000 units per ml. of diluted semen), while the amounts of lactic acid which accumulated were not significantly affected. Addition of penicillin increased the percentage of glucose utilized which was recovered as lactic acid.

5. Penicillin retarded bacterial growth at all levels in both freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was found in the semen without penicillin. The initial plate counts for the five ejaculates studied ranged from 200 to 960,000 bacteria per ml. of undiluted semen, with a mean of 364,000 bacteria per ml.

6. There was no appreciable loss in penicillin activity in diluted semen stored for 8 days and only a slight decrease in concentration after storage for 16 days at 4.5° C.

## REFERENCES

- (1) ABRAHAM, E. P., AND CHAIN, E. An Enzyme from Bacteria Able to Destroy Penicillin. *Nature*, 146: 837. 1940.
- (2) BARKER, S. B., AND SUMMERSON, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. *J. Biol. Chem.*, 138: 535-554. 1941.
- (3) IVANOV, E. Method of Rendering Infected Mammalian Sperm Incapable of Transmitting Infection. *Compt. rend. soc. biol.*, 80: 765-767. 1917. (*Chem. Abs.*, 12: 47-48. 1918).
- (4) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (5) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (6) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (7) SCHMIDT, W. H., AND MOYER, A. J. Penicillin. I. Methods of Assay. *J. Bact.*, 47: 199-209. 1944.
- (8) SHETTLES, L. B. Resistance of Human Spermatozoa *in vitro* to Sulfanilamide and Sulfapyridine. *Proc. Soc. Exptl. Biol. Med.*, 44: 392-395. 1940.
- (9) SOMOGYI, M. Determination of Blood Sugar. *J. Biol. Chem.*, 160: 69-73. 1945.





## THE EFFECT OF CONDITIONS OF STORAGE ON THE VISCOSITY OF SWEETENED CONDENSED MILK

B. H. WEBB AND C. F. HUFNAGEL

*Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture*

The viscosity of sweetened condensed milk increases with age until a gel structure develops. The time required for the milk to change from a fluid to a gel state depends on the quality of the raw milk, the manufacturing processes employed, the composition of the finished milk, and the conditions of storage to which the product is subjected. This paper is concerned with the effect on viscosity of variations in storage conditions, especially the time and temperature of storage.

Sweetened condensed milk should be smooth and free-flowing, but viscous enough to prevent settling of lactose and rise of fat during storage. When age thickening proceeds to the stage of gelation, the milk is no longer suitable for many food uses. Deterioration in flavor generally accompanies the change in viscosity. The data in this report indicate some of the storage conditions that retard undesirable increases in viscosity.

### EXPERIMENTAL PROCEDURE

The preparation of laboratory samples of sweetened condensed milk that were uniform in viscosity from batch to batch and from day to day was found to be very difficult. After extensive trials a technique was developed for the processing of 100 lb. of milk of 3.8 per cent fat and 9.15 per cent solids-not-fat, to which was added during concentration 18 lb. of sugar as a boiled sirup. All temperatures were controlled carefully, and the time required to perform each operation was the same for every batch of milk. The milk was forewarmed in a steam-jacketed kettle and concentrated in a 28-inch vacuum pan equipped with a steam jacket for finishing small batches of milk. Cooling and crystallizing were done in a water-jacketed vessel equipped with a stirrer.

Despite the precautions that were taken in preparing laboratory samples, these age thickened more rapidly than did the commercial milks. The laboratory samples were used chiefly in preliminary tests. The results obtained with the experimental milks differed from those with the commercial products in the magnitude of their viscosities, but the relationship between viscosity and the factors being studied was approximately the same in both types of samples.

The commercial sweetened condensed milk was prepared as parts of regular runs in a large condensery in northern New York. Grade A raw

Received for publication September 20, 1947.





## THE EFFECT OF CONDITIONS OF STORAGE ON THE VISCOSITY OF SWEETENED CONDENSED MILK

B. H. WEBB AND C. F. HUFNAGEL

*Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture*

The viscosity of sweetened condensed milk increases with age until a gel structure develops. The time required for the milk to change from a fluid to a gel state depends on the quality of the raw milk, the manufacturing processes employed, the composition of the finished milk, and the conditions of storage to which the product is subjected. This paper is concerned with the effect on viscosity of variations in storage conditions, especially the time and temperature of storage.

Sweetened condensed milk should be smooth and free-flowing, but viscous enough to prevent settling of lactose and rise of fat during storage. When age thickening proceeds to the stage of gelation, the milk is no longer suitable for many food uses. Deterioration in flavor generally accompanies the change in viscosity. The data in this report indicate some of the storage conditions that retard undesirable increases in viscosity.

### EXPERIMENTAL PROCEDURE

The preparation of laboratory samples of sweetened condensed milk that were uniform in viscosity from batch to batch and from day to day was found to be very difficult. After extensive trials a technique was developed for the processing of 100 lb. of milk of 3.8 per cent fat and 9.15 per cent solids-not-fat, to which was added during concentration 18 lb. of sugar as a boiled sirup. All temperatures were controlled carefully, and the time required to perform each operation was the same for every batch of milk. The milk was forewarmed in a steam-jacketed kettle and concentrated in a 28-inch vacuum pan equipped with a steam jacket for finishing small batches of milk. Cooling and crystallizing were done in a water-jacketed vessel equipped with a stirrer.

Despite the precautions that were taken in preparing laboratory samples, these age thickened more rapidly than did the commercial milks. The laboratory samples were used chiefly in preliminary tests. The results obtained with the experimental milks differed from those with the commercial products in the magnitude of their viscosities, but the relationship between viscosity and the factors being studied was approximately the same in both types of samples.

The commercial sweetened condensed milk was prepared as parts of regular runs in a large condensery in northern New York. Grade A raw

Received for publication September 20, 1947.

milk was used, and all manufacturing operations were conducted according to the best factory practice. The finished product was representative of the highest commercial grade of sweetened condensed milk. The condensed milk was shipped from the plant to the Bureau laboratories by express, the trip requiring 5 or 6 days.

The milks were stored in rooms where the temperature was controlled so that fluctuations did not exceed  $\pm 2^{\circ}$  F. Samples were not moved or disturbed during storage.

Reliable viscosity determinations on sweetened condensed milk are difficult to make. Stebnitz and Sommer (6) constructed a special viscosimeter utilizing the falling-sphere method. The measurements reported here were made under carefully controlled conditions with a McMichael viscosimeter and standardized wires. The determinations were made at  $86^{\circ}$  F. in a room maintained at this temperature. Each determination was made with a new sample of milk transferred in the same way and without stirring to the viscosimeter cup. Measurements often were made on duplicate samples and sometimes five or six samples were used to establish a single value. All milk samples either had an initial viscosity or soon developed a viscosity high enough to prevent lactose and fat separation during storage. It is believed that the viscosity values obtained during the work reflect the body condition the consumer would find in the milk. The viscosity is reported in poises but, because of the nature of the material, the values are relative rather than absolute.

Consideration was given to the possibility of bacterial growth during storage of the sweetened condensed milk. Rice and Downs (4) found that most of the organisms that might cause age thickening grew when the sugar ratio was less than 62.5  $\left( \text{sugar ratio} = \frac{\% \text{ sugar}}{\% \text{ sugar} + \% \text{ water}} \times 100 \right)$  but that growth sometimes occurred up to 64.5. The increase in titratable acidity found by Rice and Downs to accompany bacterial growth was 0.2 to 0.6 per cent after about 30 to 60 days of incubation.

The sugar ratio of the milks used in these experiments was 62.5 to 63.0. The titratable acidity of the samples stored at various temperatures was not always determined, but the tests that were made showed that the acidity of the milks increased about 0.1 per cent during a 3- or 4-month period at the higher temperatures. The age thickening observed in the sweetened condensed milks discussed in this paper was not considered to be influenced significantly by bacterial changes.

#### RESULTS

Cooling of the product and crystallization of the lactose in sweetened condensed milk may require 2 or 3 hours at temperatures that affect the viscosity of the milk. The effect of the rate of cooling sweetened condensed milk on

its age thickening is shown in table 1. The data represent the average values from five experiments with five different batches of milk. After the concentrates were dropped from the pan, they were cooled to 86° F. in 10 to 15 minutes and seeded. The cooling process of the rapidly cooled samples was continued, but the slowly cooled concentrates were held and stirred at 86° F. for about 2 hours before cooling was continued. The results indicate that the rate of age thickening is substantially the same for both methods of cooling. The lactose crystals in the rapidly cooled milk were a little larger (about 16  $\mu$ ) in size than the lactose crystals in the slowly cooled milk (about 12  $\mu$ ).

The relationship between container size and the age thickening of sweetened condensed milk was investigated. The experiments were done on fresh, commercially manufactured samples that were received in the Bureau

TABLE 1

*The effect of rate of cooling on age thickening of sweetened condensed milk\**

Storage time at 86° F.	Viscosity after storage	
	Cooled in 162 min. to 63° F.	Cooled in 38 min. to 52° F.
(days)	(poises)	(poises)
1	115	110
4	204	193
12	365	362
24	583	550

\* The data represent average values from 2 skim and 3 whole sweetened condensed milks. The samples were made in the Research Laboratory pilot plant during March and April. After condensation, each batch was divided into two parts, one for slow and the other for rapid cooling. The cooled milk was canned and placed at once in a storage room at 86° F.

laboratories in 30-gallon tight oak barrels. Smaller containers in the form of cans of various sizes were filled with sweetened condensed milk. About 3 gallons of milk was taken from the test barrels for this purpose. During the storage period one of the small cans of milk was opened for each viscosity determination.

The milk in the barrels was sampled through the bungs by means of a 1-inch diameter metal tube inserted diagonally from the bung toward one end of the barrel. Care was taken to close the bungs tightly after sampling. Table 2 shows some results of the study of the effect of container size on the viscosity of sweetened condensed milk. The tight oak barrels of sweetened condensed milk were held on the bilge with bungs down.

Two barrels of the milk received in September, 1944, were put in storage at 86° F. Samples were obtained from one barrel while the other was held unopened until the end of the test. When the sealed barrel was opened at the bung after 76 days, there was insufficient oxygen in the headspace to



support a match flame. The average viscosity of the milk (380 poises) and the titratable acidity (0.55 per cent) were substantially the same as those of the milk in the barrel that had been used for sampling (table 2).

The viscosity of the milk in the barrel that was not opened for sampling was different in various parts of the barrel, after 76 days at 86° F., being 517 poises at the surface and 478 poises at the bottom. In several places near the center of the barrel the viscosity of the milk was 352 poises. When the barrel was filled by the manufacturer, the temperature of the milk was about 50° F., and the milk in the center of the barrel probably required appreciable time to warm to 86° F. In addition, some surface thickening

TABLE 2  
*Effect of container size on the viscosity of two lots of sweetened condensed milk of commercial manufacture*

Storage time (days)	Barrel (30 gal.) (poises)	No. 3 can (poises)	No. 1 can (poises)	Baby can (poises)
Milk of September 27, 1943*—storage temperature 70° F.				
1 .....	69	.....	69	69
22 .....	70	.....	78	81
40 .....	92	.....	111	115
92 .....	140	.....	.....	182
180 .....	148	.....	233	255
256 .....	246	.....	285	346
357 .....	368	.....	446	550
Milk of September 26, 1944†—storage temperature 86° F.				
1 .....	39	39	39	39
8 .....	64	88	86	86
17 .....	80	101	108	119
35 .....	143	168	176	176
74 .....	385	407	429	429

Composition figures furnished by the manufacturer:

\* 9.25% fat, 23.0% M.S.N.F., 42.75% sucrose, 25% water.

† 8.73% fat, 23.62% M.S.N.F., 42.75% sucrose, 24.9% water.

Sugar ratio of these milks = 63.

may have been caused when this barrel dried and leaked slightly between the staves. This occurred in the middle of the storage period but the staves tightened up in 3 days when the humidity in the storage room was raised.

The effect of time and temperature of storage upon the viscosity of sweetened condensed milk was investigated. Samples were prepared June 1, 1945, by a cooperating manufacturer as part of a large commercial batch. The samples contained 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent moisture. A barrel of this sweetened condensed milk was shipped to a canning plant, where it was re-packed in special 2-ounce cans on June 8. Four hundred of these small cans were received in these laboratories June 21 and stored at six different tempera-

tures. Each can held enough milk to make one viscosity determination. Some of the results are plotted in figure 1.

This milk showed a large increase in acidity with age, but apparently this was not caused by bacterial growth. The figures determined on the manufacturer's milk in six of the 2-ounce cans showed an average standard plate count of 4,380 and a titratable acidity of 0.43 per cent. After 640

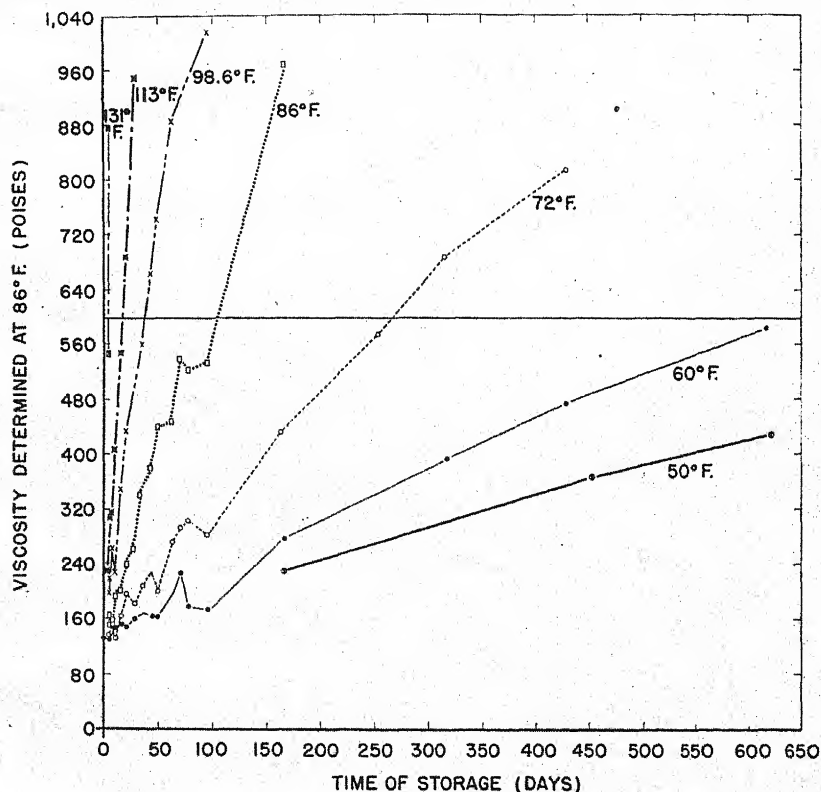


FIG. 1. The effect of time and temperature of storage on the viscosity of sweetened condensed milk stored in 2-ounce cans. All samples were from the same batch of milk, but the samples used for storage at 50° F. were delayed 2 weeks in transit at summer temperatures, causing an undue initial increase in viscosity. The composition of the milk was 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent water.

days of storage at different temperatures, the titratable acidity and the acid intensity of this milk were: 50° F. storage, 0.84 per cent and pH 6.01; 60° F. storage, 0.85 per cent and pH 6.09; 72° F. storage, 1.0 per cent and pH 5.90. No signs of bacterial growth were found in samples held at these temperatures for 640 days.<sup>1</sup> Anaerobe tubes, direct smears, and standard

<sup>1</sup> Bacteriological examination of this milk was made by Harold R. Curran of these laboratories.

plates were practically negative. While it was not shown that growth of organisms was absent during the whole storage period, the available evidence indicates that the acidity increase was due to chemical changes rather than to bacterial activity.

The storage temperature of a group of the 2-ounce cans of sweetened condensed milk was varied from 60° F. to 98° F. by shifting the cans every 24 hours from one temperature to the other. The initial viscosity of the milk was 132 poises, but after storage for 170 days a soft gel had formed with a viscosity of 1,155 poises. By reference to figure 1 it may be determined that this is the viscosity the milk would have reached if it had been held 170 days at a constant temperature of 89° F.

Sweetened condensed milks that were stored for several months at 0° F. did not show important physical changes. There was no measurable change in viscosity and no apparent change in the dispersion of the protein or the fat. There was no protein flaking or insolubility, such as occurs in frozen unsweetened milk. There was an increase in the size of lactose crystals in sweetened condensed milks held at 0° F. only when the lactose was incompletely crystallized as a result of improper cooling during the manufacturing process.

#### DISCUSSION

The keeping quality of sweetened condensed milk is closely associated with its viscosity. Whenever sweetened condensed milk age thickened to a viscosity of 600 to 800 poises, it generally was no longer suitable for use in high-grade food products. Off flavors often developed and the milk was too viscous to handle easily. A soft gel structure was present at 1,000 poises. The gel could be reduced by stirring, but it re-formed when the milk remained undisturbed.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under the same conditions of temperature.

The data of table 2 indicate that sweetened condensed milk packed in barrels will remain fluid a little longer than milk packed in cans when both are held under the same conditions of storage. The temperature of milk in cans follows fluctuations in storage temperature more closely than does the temperature of milk in barrels. Commercial experience indicates that the sweetened condensed milk in the middle of a 50-gallon barrel will require about 7 days to reach 85° F. after previous storage at about 60° F. (1). A longer time is required for the reverse change to take place. Cool milk packed in barrels will remain cool and thin longer after the container is placed at a high temperature than will milk in small cans.

These considerations, and the fact that high sugar concentrations stabilize the milk and retard viscosity changes, support the suggestion (2)



that bulk sweetened condensed milk may have a minimum sugar ratio of 60, while the sugar ratio of the canned milk should be at least 62.5. However, a bulk sweetened condensed milk with a sugar ratio of 62.5 will be superior to one that contains less sugar.

Data from the curves of figure 1 were used to prepare figure 2, in which the logarithms of the viscosities are plotted against the temperatures of storage.

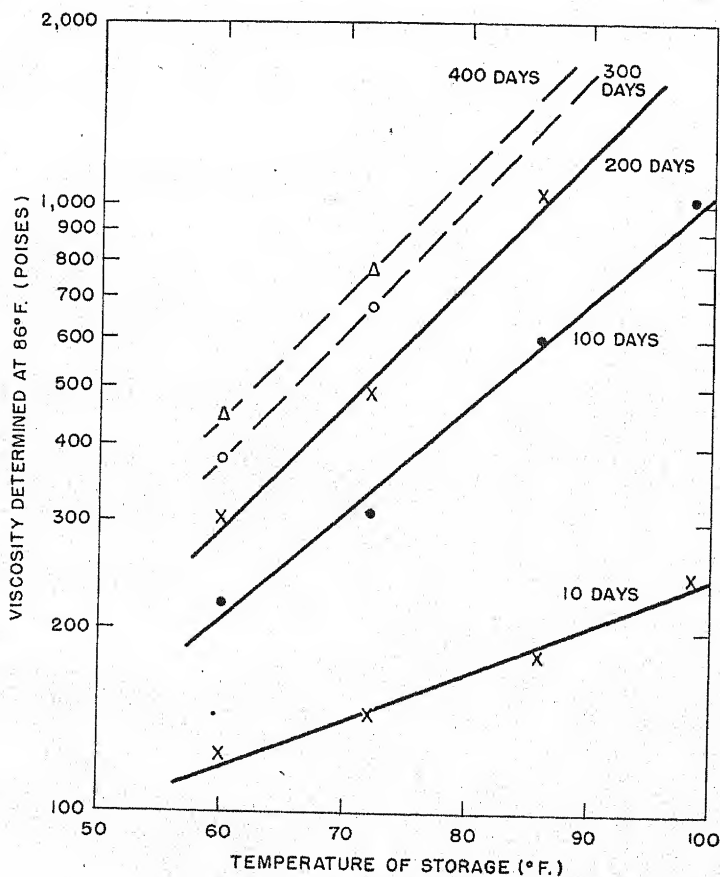


FIG. 2. The effect of time and temperature of storage on the viscosity of sweetened condensed milk. Data taken from figure 1.

The time required for the sweetened condensed milk to reach a viscosity of 600 poises at different temperatures is indicated in figure 1. When these temperature data were plotted against the logarithms of the time of storage, straight line no. 1, figure 3, was obtained. Values for 30, 40, and 50° F., obtained by extrapolation of curve no. 1, figure 3, are 4,950, 2,460, and 1,225 days, respectively. Other data also are plotted on figure 3.

If  $n$  = viscosity and  $c, c', c''$  = constants, the relationship shown in figure 1 may be expressed as follows:  $n = c'$  (time of storage) or  $\log n = c''$  (log time), while for figure 2  $\log n = c$  (temperature of storage); then  $c$  (temperature) =  $c''$  (log time), the relationship shown in figure 3 where the viscosity is constant.

The data given in figure 1 and re-plotted in figures 2 and 3 show that

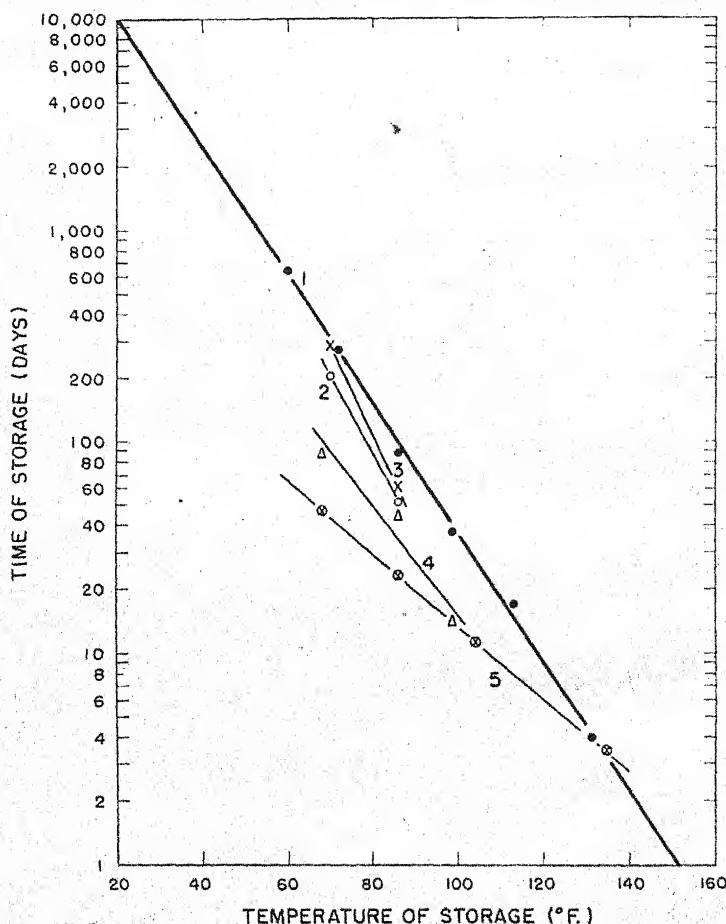


FIG. 3. The relationship between the time and temperature of storage in the development of viscosity of sweetened condensed milk: Curve 1, data from figure 1, viscosity of 600 poises. Curve 2, data from table 2, viscosity of 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in baby-size cans. Curve 3, data from table 2, viscosity equals 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in barrels. Curve 4, data from Rogers *et al.* (5), viscosity of 12° rotation on special viscosimeter. Curve 5, data from Leighton and Mudge (3) in which the time is given in hours (not days) required for the milk to start thickening.

the viscosity of sweetened condensed milk increases arithmetically with time of storage but logarithmically with temperature of storage. For conditions of constant viscosity the time of storage varies logarithmically with the temperature of storage.

Although the logarithms of the storage times required for a sweetened condensed milk to reach a certain viscosity, plotted against the temperatures of storage, yield a straight line (fig. 3), the slope of this line may vary with different milks. The milk used by Leighton and Mudge (3), figure 3, gave a line of different slope from that of the other milks. The data of Leighton and Mudge were obtained by observing the number of hours required for the milk to start thickening. It seems probable that most commercial milks will thicken much as did milk no. 1 of figure 3. It should be possible to estimate the length of time a sweetened condensed milk will retain a satisfactory viscosity at various storage temperatures by using the data of figure 3. The rate of thickening approximately doubles with each increase of 10° F. between 30 and 60° F.

In some cases the slope of the time-temperature curve of a milk may differ from that of curve 1, figure 3. A line may be drawn for any milk if two points are obtained. To secure these points quickly, about six samples of a milk may be held at each of two high temperatures such as 98° and 120° F. Viscosity determinations should be made at 1- or 2-day intervals and curves drawn like those of figure 1. Two points having the same viscosity value then may be used to construct a time-temperature curve patterned after that of figure 3. Other points may be taken from the curve or they may be calculated from the geometric equation for a straight line. The equation may be stated in the following form:

$$\frac{\log y - \log y_1}{\log y_2 - \log y_1} = \frac{x - x_1}{x_2 - x_1}$$

when

$y$  = storage time

$x$  = storage temperature

$y_1$  and  $y_2$  = storage times at  $x_1$  and  $x_2$  storage temperatures. For a milk similar to that shown in curve 1, figure 3, the storage time ( $y$ ) required to reach a viscosity of 600 poises at a given temperature ( $x$ ) may be found from the equation calculated from the plot:

$$\log y = 4.64 - \frac{1.82 x}{59}$$

It is important to note that the time-temperature relationship of figure 3 will hold only when the storage temperature is constant. If the storage temperature fluctuates, the changes in viscosity will be dependent upon the extent of the changes.

The theoretical freezing point of sweetened condensed milk is about 5° F., but only a few ice crystals form at this temperature. Since all the



moisture in sweetened condensed milk will freeze only at a much lower temperature, neither the milk nor the container is damaged by storage at temperatures considerably below 0° F. Sweetened condensed milk that was held at 0° F. for many months did not change measurably in viscosity. Extrapolation of Curve 1, figure 3, indicates that 24,000 days (65 years) at 0° F. would be required for this milk to reach a viscosity of 600 poises.

#### SUMMARY

The viscosities of sweetened condensed milks held in the same storage room increased a little more rapidly in the milks packed in small cans than they did in the milks packed in barrels.

The viscosity of sweetened condensed milk increased logarithmically with increases in storage temperature and arithmetically with increases in storage time. For conditions of constant viscosity, time varied logarithmically with temperature. Viscosity values may be predicted by applying this relationship to time-temperature data from high temperature, accelerated storage tests.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under identical conditions of time and temperature.

The authors wish to express their appreciation to P. L. Haymes, H. D. Wilder, and A. R. Davis, of the United Milk Products Company, for their numerous suggestions and their active interest in this project and for their manufacture of the commercial samples of sweetened condensed milk.

#### REFERENCES

- (1) HAYMES, P. L. Cleveland, Ohio. Information received March, 1947.
- (2) HUNZIKER, O. F. LaGrange, Ill. Information by correspondence, April, 1947.
- (3) LEIGHTON, A., AND MUDGE, C. S. On the Endothermic Reaction Which Accompanies the Appearance of a Visible Curd in Milks Coagulated by Heat: A Contribution to the Theory of the Heat Coagulation of Milk. *J. Biol. Chem.*, 56: 53-73. 1923.
- (4) RICE, F. E., AND DOWNS, P. A. Sweetened Condensed Milk. I. Bacterial Thickening. *J. Dairy Sci.*, 6: 532-548. 1923.
- (5) ROGERS, L. A., DEYSHER, E. F., AND EVANS, F. R. Factors Influencing the Viscosity of Sweetened Condensed Milk. *J. Dairy Sci.*, 3: 468-485. 1920.
- (6) STEBNITZ, V. C., AND SOMMER, H. H. The Age Thickening of Sweetened Condensed Milk. I. Seasonal Variations. *J. Dairy Sci.*, 18: 757-768. 1935.

## DETERMINATION OF CHEESE LIPASE<sup>1</sup>

MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE

*The Departments of Biochemistry and Dairy Industry, College of Agriculture,  
University of Wisconsin, Madison*

In recent years lipolysis has been directly associated with flavor development in Cheddar cheese. In order to study directly the fat breakdown in Cheddar cheese, procedures for the quantitative determination of the lipolytic agents responsible are necessary.

A method for the determination of milk lipase previously was reported by the present authors (1). Since no methods for the quantitative determination of cheese lipase have been described, it was necessary to devise one. In the present paper such a method is presented. It is similar in principle to the milk lipase determination previously reported (1) and, similarly, has the limitation that it measures only enzymes capable of hydrolyzing tributyrin.

### EXPERIMENTAL

*Effect of pH on cheese lipase activity.* In figure 1 the pH activity curve for the hydrolysis of tributyrin by cheese extracts is given. Determinations of enzyme activity at various pH values were carried out according to the procedure subsequently outlined under "Quantitative Determination of Cheese Lipase" with the following exception: In place of 2 ml. of 0.38 molar aniline buffer, 2 ml. of a 0.38 molar aniline, 0.38 molar phosphate buffer were added. By means of this composite buffer, pH was controlled throughout the pH range desired.

It will be seen from the figure that tributyrin is split most rapidly at pH 5. There also is a secondary optimum at pH 6.5 to 7.0. This secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the lipolytic activity seems quite constant, however, throughout the cheese-ripening period. The presence of two peaks in the pH optimum curve of cheese lipase indicates strongly that two or more lipases are present. The enzymes active at pH 6.5, however, are not active in ripening cheese, since the pH of the cheese seldom rises above 5.4. Therefore, only the lipolytic activity at pH 5 was considered important.

*Effect of buffers on cheese lipase activity.* In the development of a method for the estimation of cheese lipase, it was necessary to find a buffer which did not affect the lipase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese lipase. As may be seen, the citrate, acetate, and

Received for publication September 22, 1947.

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Cheese Institute.

phthalate buffers had an inhibitory effect on the lipase. None of the other three buffers inhibited the enzyme. Any buffer containing the carboxyl group seems to inhibit the lipase. The flattening of the curves in figures 3 and 4 when a titration greater than 0.40 ml. of 0.1 N NaOH is obtained per 5 ml. of incubation mixture probably is due to reversible combination of the butyric acid with the enzyme to form an enzyme-product complex. The buffers containing carboxyl groups probably inhibit the lipase in the same way. The phosphate buffer was not used in the cheese lipase method as

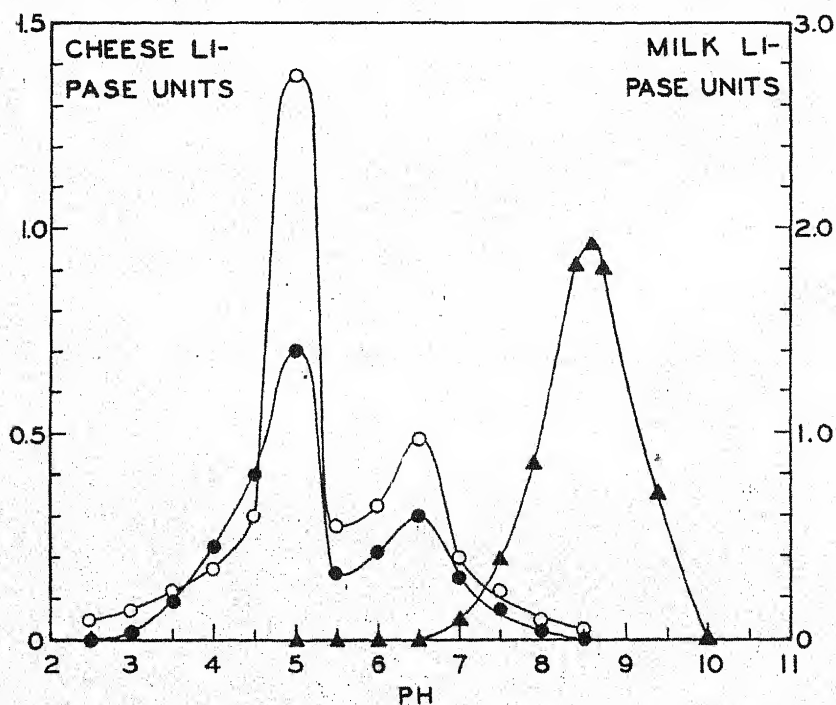


FIG. 1. Effect of pH on rate of tributyrin hydrolysis by cheese extracts. The effect of pH on the rate of tributyrin hydrolysis by milk lipase is presented for comparison. Triangles represent milk lipase, dots represent 2-month-old raw milk cheese, and circles represent 10-month-old raw milk cheese.

finally adopted because it has very little buffering capacity at pH 5. Both the pyridine and aniline buffers have good buffering capacity at pH 5. Since the aniline buffer was the more convenient to use, it was chosen for use in the final method.

*Effect of activators on cheese lipase activity.* Various compounds known to possess definite activating properties for other enzymes were tested for activating effect on cheese lipase. As may be seen in table 1, none of them gave activation; most of them were inhibitors.



TABLE 1  
The effect of activators and buffers on cheese lipase activity

Substance used	Concentration	Apparent cheese lipase in sample
	Activators	(units/ml.)
None .....	.....	0.88
Zinc chloride .....	0.001	0.82
Potassium cyanide .....	0.001	0.64
Manganous sulphate .....	0.001	0.80
Magnesium chloride .....	0.001	0.88
Calcium chloride .....	0.001	0.90
Cysteine .....	0.001	0.78
	Buffers	
Aniline .....	0.0625	0.70*
Pyridine .....	0.1	0.70
Phosphate .....	0.1	0.70
Citrate .....	0.1	0.16
Phthalate .....	0.1	0.22
Acetate .....	0.1	0.34

\* The lipase solution used in the buffer experiments was not the same as that used in the activator experiments.

*Variation of hydrolysis rate with amount of tributyrin present.* Figure 2 shows that the rate of hydrolysis of tributyrin by cheese lipase is affected by concentrations of tributyrin up to 1.5 per cent. At this level and above, the hydrolysis rate appears to be constant.

*Kinetics of cheese lipase.* In figure 3 the relation between quantity of cheese lipase and tributyrin hydrolysis is presented. It may be seen that the tributyrin hydrolysis obtained is directly proportional to the quantity of cheese lipase present, up to the point when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

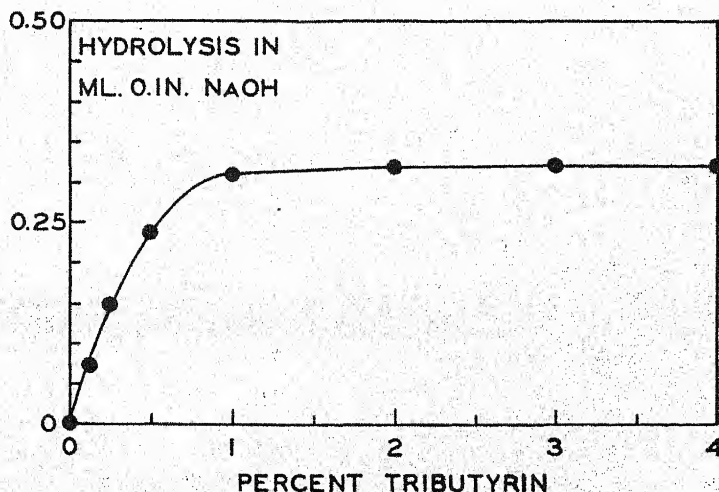


Fig. 2. Variation of hydrolysis rate with amount of tributyrin present.

In figure 4 the effect of incubation time on tributyrin hydrolysis by cheese lipase is shown. It may be seen that the hydrolysis of tributyrin by cheese lipase at pH 5 is linear up to the time when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

The enzyme unit used in the cheese lipase determination is based on the relationships presented in figure 3:

#### QUANTITATIVE DETERMINATION OF CHEESE LIPASE

In the method finally adopted, the following procedure is used. Twenty grams of the cheese sample to be tested are weighed out with an accuracy of  $\pm 0.1$  g. To the weighed sample in a Waring Blender or other suitable

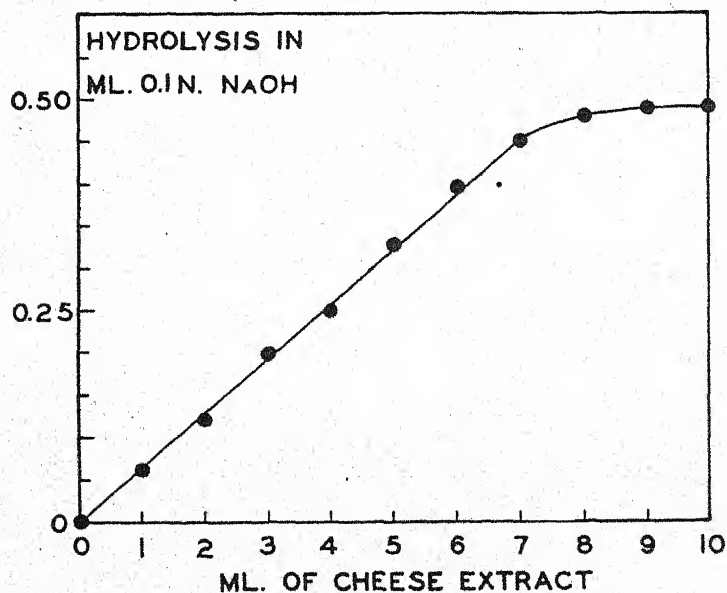


Fig. 3. Relation between quantity of cheese lipase and amount of tributyrin hydrolysis.

mixer, enough distilled water is added to make a total volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set to high speed and allowed to run 7 more minutes. At the end of this time the mixture is in the form of a smooth, white suspension. This suspension is transferred quantitatively to a small hand homogenizer using a minimum amount of distilled water. The suspension then is run five to six times through the homogenizer, which further insures complete extraction of the enzyme. After quantitative transfer to a 250-ml. centrifuge tube, the suspension is centrifuged for 10 minutes at 2,000 r.p.m. At the end of this period the solid material in the suspension will have settled out, leaving a faintly turbid supernatant which is drawn off. The residue is washed care-

fully and centrifuged at 2,000 r.p.m. for 10 minutes with three successive 20 ml. portions of distilled water, which all subsequently are added to the turbid supernatant. The supernatant plus the three washings is diluted to a total volume of 200 ml. and the resulting cheese extract is used directly as the enzyme sample to be analyzed. Experiments have shown that all the lipolytic activity present in the cheese is washed free from the cheese curd by the above procedure.

For the analysis, a suitable volume (depending upon its activity) of the

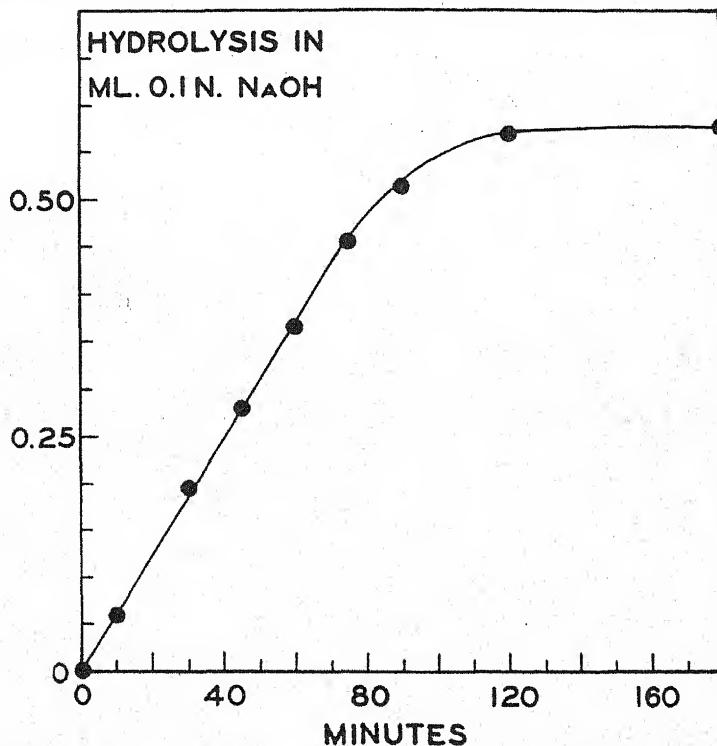


FIG. 4. Effect of incubation time on tributyrin hydrolysis by cheese lipase.

cheese extract is added to 2 ml. of 0.38 molar aniline buffer at pH 5. This solution is diluted to a volume of 12 ml. with distilled water and placed in a water bath held at 40° C. After the solution has attained this temperature, 0.2 ml. of tributyrin is added, making a total volume of 12.2 ml. The mixture is shaken vigorously for 1 minute, and a 5-ml. aliquot immediately is removed for titration. After an incubation period of 60 minutes<sup>2</sup> at 40° C., the tube and its contents are shaken for 30 seconds and another 5-ml. aliquot is titrated. The difference in titration between the two aliquots

<sup>2</sup> In all the experimental work presented, incubation times of 60 minutes were used unless otherwise specified.



represents tributyrin hydrolysis. The titration is carried out as follows: The 5-ml. aliquot is pipetted into a 50-ml. Erlenmeyer flask containing 5 ml. of a 0.02 per cent solution of thymolphthalein in 95 per cent alcohol. After addition of 2 ml. of ether, the solution is titrated with 0.1 N alcoholic NaOH from a burette calibrated at 0.01-ml. intervals. Titration is carried to a definite blue color. It is convenient to prepare an artificial endpoint comparison flask containing a dilute aqueous solution of  $\text{CuSO}_4$  and  $\text{CoCl}_2$  to which enough alumina cream has been added to give a close resemblance to the actual titration flask.

One cheese lipase unit is defined as the amount of enzyme which, when diluted to 12.2 ml. and incubated for 60 minutes under conditions of the determination as described above, will give a titration increase of 0.1 ml. of 0.1 N NaOH for a 5-ml. aliquot. For instance, if 4 ml. of cheese extract

TABLE 2

*Comparison of volatile acid distillate titration differences and direct titration differences*

Enzyme sample no.	Amount of hydrolysis of tributyrin by titration differences of volatile acid distillates	Amount of hydrolysis of tributyrin by direct titration differences
	(ml. 0.1 N NaOH)*	(ml. 0.1 N NaOH)*
A-1	0.05	0.05
A-2	0.36	0.34
A-3	0.42	0.43
A-4	0.17	0.19
A-5	0.27	0.27
A-6	0.12	0.11

\* All values expressed as ml. 0.1 N NaOH represent the amount of hydrolysis of tributyrin by various dilutions of cheese enzyme sample A.

are used, and the observed titration increase is 0.36 ml. of 0.1 N NaOH, the extract contains 0.9 enzyme unit per ml. The linear relation here assumed between titration increase and amount of enzyme is shown to be justified by figure 3 and table 3.

If the observed titration increase is more than 0.40 ml., the determination should be repeated with a smaller volume of cheese extract. If the titration increase is less than 0.1 ml., a larger volume should be used. Cheese extracts have been found to retain their activity for at least 10 hours in the refrigerator.

#### RELIABILITY OF THE METHOD

Evidence that the titration used measures only glyceride hydrolysis is afforded by table 2, where direct titration figures are compared with titrations of volatile acid distillates of equivalent aliquots. Good agreement was obtained in all cases. The volatile acid distillates were obtained as follows: A 5-ml. aliquot of the incubation mixture was pipetted into the distillation

flask. The pH immediately was adjusted to 2 (red to thymol blue) with 5 N  $\text{H}_2\text{SO}_4$ . A constant volume distillation then was carried out until about five times the volume of the original sample had been distilled over. The distillates obtained were titrated to the phenol red endpoint.

The reliability of the method and of the standard curve also was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 3. As may be seen, determinations agreed within 10 per cent.

TABLE 3  
*Effect of sample size on apparent cheese lipase content of a cheese extract*

Sample size	Titration differences	Lipase content
(ml.)	(ml. 0.1 N NaOH)	(units/ml.)
1.0	0.07	0.70
1.0	0.08	0.80
3.0	0.23	0.77
3.0	0.23	0.77
5.0	0.38	0.76
5.0	0.39	0.78
6.0	0.45	0.75
6.0	0.46	0.77
7.0	0.47	0.67

#### DISCUSSION

It should be emphasized that the method outlined here has a number of failings. Since the lipolytic activity of Cheddar cheese undoubtedly is due to a mixture of lipases produced by the mixed flora of the cheese, any method of quantitative estimation will give consistent results only if the ratio in which the various lipases are present remains unchanged. This is true because various substrates are attacked at different rates by different enzymes. If a mixed substrate such as butterfat is used, the determination does not inform us whether the fatty acids liberated are the highly flavored volatile fatty acids or the relatively tasteless higher fatty acids. On the other hand, if a pure substance is used as a substrate, the determination can measure only activity toward this substrate, and again no direct information is obtained regarding liberation of the particular acids of interest to the investigator.

Any lipase determination will measure only lipases active at the pH of the determination. The present determination will not detect the presence of lipases active only at high pH values, since the determination is designed to measure only those enzymes active at pH 5.

#### SUMMARY

1. A method is presented for the quantitative determination of lipolytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

2. Hydrolysis of tributyrin by the Cheddar cheese lipase system is most rapid at pH 5. A secondary optimum occurs at pH 6.5 to 7.0.

#### REFERENCE

- (1). PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Milk Lipase. J. Dairy Sci., 26: 233-240. 1943.



## LIPASE ACTIVITY DURING MAKING AND RIPENING OF CHEDDAR CHEESE<sup>1</sup>

MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE

*The Departments of Biochemistry and Dairy Industry, College of Agriculture,  
University of Wisconsin, Madison*

In recent years it often has been assumed that rancidity in cheese is due to the production of lower fatty acids such as butyric acid by the action of milk lipase on natural milk fats. It first was suggested by Rice and Markley (18) that milk lipase was one of the causes of rancidity in cheese. Lane and Hammer found that: (a) typical rancid Cheddar cheese could be produced experimentally by the addition of sources of lipase, such as rennet paste (11) and pancreatin (13), to cheese milk, and (b) typical rancid Cheddar cheese could be produced experimentally by the addition of homogenized raw cream to raw skim milk as a means of milk lipase activation (12). Hood *et al.* (10) confirmed the reports of Lane and Hammer and further found that typical rancid Cheddar cheese could be produced experimentally under commercial conditions by vigorous agitation of raw cheese milk at various temperatures (7, 8). Since these earlier publications, Hlynka and Hood (2, 3), Hlynka *et al.* (5, 6, 9), and others (1, 14) have reported a great deal of work which has been carried out on the basis of the milk lipase theory. A large part of this work has been carried out with commercial lipases or crude enzyme preparations as the source of experimental lipase. There is no reason to believe, however, that the lipases from these sources have properties comparable to milk lipase. Moreover, commercial lipase preparations and crude enzyme preparations generally contain many other types of enzymes as impurities.

In a brief preliminary report (15) Peterson and Johnson pointed out that milk lipase is inactive at the pH of Cheddar cheese and is completely absent from Cheddar cheese after pressing. The data on which these conclusions were based are presented in this paper. In a recent paper, Hlynka and Hood (4) also concluded that milk lipase is inactive in Cheddar cheese after it is made.

The purpose of the present paper is to present studies of the differences in cheese lipase content during the making and ripening of raw and pasteurized Cheddar cheese from the same milk. In the preliminary report (15) it was mentioned that lipolytic activity of a type different from that of milk lipase gradually appears in ripening Cheddar cheese. This

Received for publication September 22, 1947.

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Cheese Institute.

lipolytic activity has been termed "cheese lipase" by the present authors (17). Cheese lipase is distinctly different from milk lipase in its pH optimum (17) and its chemical kinetics with relation to the hydrolysis of tributyrin (16, 17).

#### METHODS

*Milk lipase.* The method for the determination of milk lipase previously reported by the present authors (16) was used for all milk lipase analyses. In this method, the degree of hydrolysis of tributyrin at 40° C. by milk lipase in a solution buffered at pH 8.5 is used as a measure of milk lipase activity.

*Cheese lipase.* The method for the determination of cheese lipase reported by the present authors (17) was used for all cheese lipase analyses. In this method the degree of hydrolysis of tributyrin at 40° C. by cheese lipase in a solution buffered at pH 5.0 is used as a measure of cheese lipase activity.

*Cheesemaking procedure.* The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. Small vats of cheese were made (460 lb. of milk). Each such vat yielded two 22-lb. cheeses. Cheeses were made by an experienced maker according to standard procedures. The green cheeses produced were kept at 68° F. for a period of 2-3 days and then were transferred to a cold room (40-45° F.) for the remainder of the ripening period.

To make both raw and pasteurized milk cheese from the same milk, 920 lb. of mixed, raw, whole milk was divided into two equal portions. The first portion received no treatment. The second portion was pasteurized by holding 30 minutes at 145° F. Each portion then was placed in a separate vat, and both received identical subsequent treatment according to standard Cheddar cheesemaking procedures.

#### A. ROLE OF MILK LIPASE IN CHEDDAR CHEESE

*Loss of milk lipase during making.* In table 1 the stability of milk lipase at various temperatures and pH values is presented. From these data it would be expected that under the pH and temperature conditions used in the making and ripening of Cheddar cheese (table 2), there is little possibility of milk lipase being present in green or ripe Cheddar cheese. Assuming it was present, milk lipase is inactive at a pH of 6.5 or lower (16, 17). Therefore, at the pH of ripening Cheddar cheese (5.0-5.4), milk lipase would have no effect even if present.

In order to obtain direct substantiation for the above conclusions, milk lipase estimations were made before, after, and, if necessary, during any operation in the making and ripening of raw milk Cheddar cheese that might affect the milk lipase content. A number of such experiments were

TABLE 1  
*Stability of milk lipase at various pH values*

pH	Per cent of lipolytic activity remaining after 30 min. incubation		
	(41° F.)	(68° F.)	(104° F.)
4.0	58.2	14.4	0
4.5	72.7	41.0	0
5.0	88.1	48.0	8.1
5.5	99.1	72.3	18.2
6.0	99.1	97.1	44.8
6.5	99.2	98.9	92.0

carried out. The results of one representative experiment are presented in table 2.

As may be seen from the table, no significant change in milk lipase content takes place until the rennet is added to the raw milk. A rapid rise in milk lipase content immediately results. The cause of this rise is not definitely known since no lipolytic activity at pH 8.5 is present in rennet

TABLE 2  
*Role of milk lipase in Cheddar cheesemaking*

Time	Temperature	pH	Source of sample	Milk lipase content*
(min.)	(° F.)			
0	55-57	6.60	Raw milk after addition to vat	29.0
13	86	6.60	Raw milk after warming to 30° C.	27.8
18	88	6.59	Raw milk after addition of starter†	27.8
73	88	6.59	Raw milk after addition of rennet‡	41.5
101	88	6.51	Coagulum after cutting of the curd	25.2
110	88		Coagulum before heating	24.0
133	104	6.48	Curd after heating to 104° F.	16.4
188	96	6.29	Curd during firming process	7.63
208	92	6.04	Curd just after dipping	1.36
208	91	6.12	Whey just after dipping	5.11
368	91	5.46	Curd just after milling	0.78
(hr.)				
24	Room temp.	5.31	Cheese just after pressing	0.00
(days)				
5	40-45	5.28	Cheese	0.00
10	40-45	5.27	Cheese	0.00

\* Milk lipase content expressed in all cases as the number of milk lipase units per gram of cheese. All figures are based on the amount of cheese obtained, which equaled 9.72 lb. per 100 lb. of milk.

† One per cent inoculum of an 18-hr. commercial starter culture added.

‡ Commercial rennet added in the amount of 10 ml. per 100 lb. milk.



alone. Since this apparent milk lipase activity disappears rapidly, it is readily seen that the factor responsible is quite unstable. There are two important inactivations or removals of true milk lipase activity. The first takes place during the heating of the coagulum to 104° F. and the holding between this temperature and 96° F. for 55 minutes. After this treatment less than one-third of the original milk lipase activity remains. This is due not only to the temperature effects but also to the low pH range encountered (6.0-6.5). The combination of these two adverse factors is more effective in the inactivation of milk lipase than either one alone. Another large loss of milk lipase occurs during the removal of whey. After dipping, less than 5 per cent of the original milk lipase remains in the curd. Only a small amount of milk lipase remains in the cheese curd just after the milling operation. Due to the low pH (5.3-5.5) of the curd, this small amount of milk lipase is rapidly inactivated, and no milk lipase activity can be found in the green cheese 24 hours after the start of making. As may be seen from the table, analyses were also run after 5 and 10 days of ripening. No milk lipase activity was found.

*Discussion.* There probably are a number of lipases present in milk. It is very probable that the present method of measuring milk lipase activity does not include all of them. It also is probable, however, that those lipases present in milk which are not capable of splitting tributyrin have little or no effect on the production from milk fat of those fatty acids which could contribute to the flavor of Cheddar cheese. The fatty acids contributing to cheese flavor probably include only those having from two to ten carbon atoms. The fatty acids containing more than ten carbon atoms have little or no taste. Since the fatty acids capable of contributing flavor to cheese must lie within this narrow range of possible number of carbon atoms, there is no reason to believe that a lipase exists in milk capable of splitting, for example, caprylic acid but not butyric acid from a glyceride ester.

As far as rancidity in cheese is concerned, there is no doubt that tributyrin is the most logical choice for a substrate in the measurement of milk lipases possibly causing the rancidity. This is true since rancidity in cheese generally is attributed to the production of lower fatty acids such as butyric acid. There is, of course, the remote possibility that a lipase present in milk would release butyric acid from mixed triglycerides and not from simple triglycerides.

#### B. LIPASE CONTENT OF CHEDDAR CHEESE AFTER MAKING

*Lipase content of raw and pasteurized Cheddar cheese.* After a ripening period of approximately 5-20 days, lipolytic activity of the cheese lipase type is found in green Cheddar cheese. In figure 1 the averages of the lipolytic activities of six pairs of pasteurized milk cheeses and raw milk

cheeses made by the procedure described under "Methods" are given. The average per cent deviation of the individual lipase analyses from the mean was less than 3 per cent.

The lipolytic activity in raw milk Cheddar cheese will be considered first. In figure 1 it will be noted that no lipolytic activity at pH 5 is present in the milk at the time it is placed in the vat. This is in accordance with data presented previously (16, 17) showing that milk lipase is inactive below pH 6.5. The first increase in lipolytic activity occurs at the time of addition of the rennet, which is about 75 minutes after the start of making. Since there is a similar increase in the lipolytic activity of corresponding

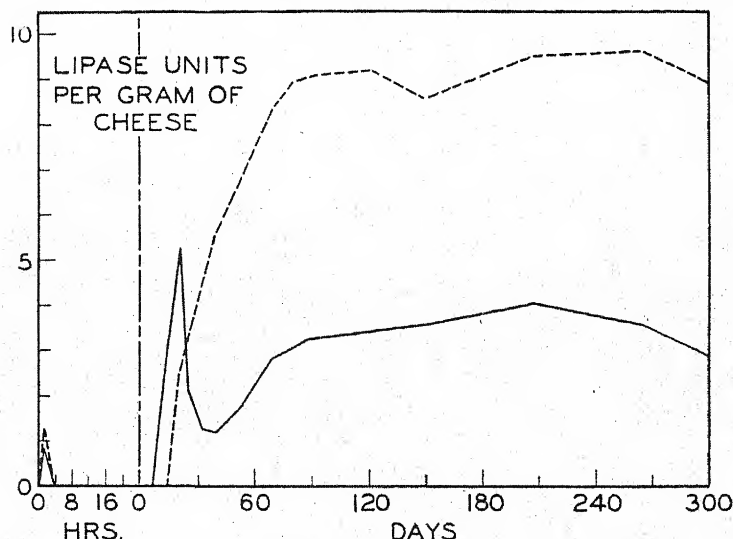


FIG. 1. Lipase activity at pH 5 in Cheddar cheese during making and a 300-day ripening period. Average values for six pairs of cheese. Solid line, pasteurized milk cheeses; broken line, raw milk cheeses.

pasteurized milk cheeses, undoubtedly this effect is caused by lipolytic enzymes which are active at pH 5 and are present in the rennet as impurities. Since this lipolytic activity attributable to rennet disappears at the time of dipping, it can have no effect on the cheese during the ripening period. After dipping there is no lipolytic activity present until after the cheese is in its final form and has been stored 5-20 days. At this time lipolytic activity is detectable, and it increases in amount steadily up to 80-90 days after making. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in large amounts at an earlier stage in the ripening period than in raw milk Cheddar cheese. The significance of this will be discussed later.

Figure 1 shows that the amount of lipolytic activity at pH 5 present during the making of the cheese is very small compared to the lip-

activity at pH 5 of the cheese during the ripening period. Therefore, even if this lipolytic activity at pH 5 present during making is carried over into the cheese in a reversibly inactivated form, its role in ripening must be small.

At most a negligible portion of the total lipolytic activity of the cheese is contributed by the milk or rennet during making. Of the possible sources of the lipolytic activity, the bacterial flora of the cheese is the most logical. No lipolytic activity is detectable in the green cheese during the first 5-6 days after making, and most of the cheese lipolytic activity is set free between the fifth and hundredth day of the ripening period after making. Since very few of the lactic acid bacteria present in Cheddar cheese are strongly lipolytic in their normal life cycles, it is suggested that the lipolytic activity of cheese at pH 5 may represent endocellular bacterial lipases of these organisms liberated by bacterial autolysis (19).

The lipolytic activity at pH 5 of pasteurized milk cheese also is presented in figure 1. No important differences in the lipolytic activities of raw and pasteurized milk Cheddar cheese during making are apparent. The first important increase in lipolytic activity for pasteurized milk Cheddar cheese during the ripening period occurs between the fifth and twentieth day after making. This increase in lipolytic activity for pasteurized milk cheese occurs considerably earlier in the ripening period than the time at which lipolytic activity is detectable in raw milk cheese. In all pairs of raw and pasteurized milk cheeses examined, the lipolytic activity in the pasteurized milk cheeses was higher on the tenth and twentieth day than in the corresponding raw milk cheeses. It is probable that the factor responsible is related to the differences in flora between raw and pasteurized milk cheese. This early lipolytic activity in pasteurized milk cheese disappears rapidly, as shown in figure 1, and the total lipase content of pasteurized milk cheese at any age after the first 30 days is less than half that of the corresponding raw milk cheese. The bacteria responsible probably are species which are greatly reduced in number during pasteurization.

Since raw milk cheese develops a higher flavor (generally attributed in large part to increased production of volatile fatty acids) than pasteurized milk cheese, it follows that lipases such as those studied in this report are at least in part responsible for more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

#### SUMMARY

1. The stability and pH activity characteristics of milk lipase are such that this enzyme can play no continuing role in the ripening of Cheddar cheese after making.



2. Analyses made at intervals during the making and ripening of raw milk Cheddar cheese show that milk lipase disappears during the making and is completely absent in the young cheese.

3. The addition of rennet extract during Cheddar cheesemaking causes an increase in lipolytic activity. This increase in lipolytic activity disappears within a period of approximately 30 minutes.

4. After 5 to 20 days, lipases which are considered bacterial begin to make their appearance in the young Cheddar cheese.

5. Most of the lipase active at pH 5 is set free between the fifth and hundredth day of ripening. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in larger amounts between the fifth and twenty-fifth day of the ripening period than in corresponding raw milk Cheddar cheese. At any time after the cheese is 30 days of age, however, the total lipolytic activity of pasteurized milk cheese is less than half that of corresponding raw milk cheese.

6. Bacterial lipases are believed to be at least in part responsible for more rapid flavor development in raw milk Cheddar cheese as compared to Cheddar cheese made from identical milk after pasteurization.

#### REFERENCES

- (1) BABEL, F. J., AND HAMMER, B. W. Fat Degradation in Cheddar Cheese Made from Pasteurized Milk, without and with Added Lipase. *J. Dairy Sci.*, **28**: 201-208. 1945.
- (2) HLYNKA, I., AND HOOD, E. G. The Reversibility of Oxidative Inactivation of Milk Lipase in Relation to Its Activity in Cheddar Cheese. *J. Dairy Sci.*, **25**: 111-115. 1942.
- (3) HLYNKA, I., AND HOOD, E. G. What Constitutes Quality in Milk for Cheddar Cheese. *Can. Dairy Ice Cream J.*, **21** (9): 19-21, 38. 1942.
- (4) HLYNKA, I., AND HOOD, E. G. Keeping Quality of Cheese with Rancid and Unelean Flavors. *Can. Dairy Ice Cream J.*, **23** (11): 35-36. 1944.
- (5) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. Effect of Proteolysis on Lipase Induced Rancidity in Cheddar Cheese. *J. Dairy Sci.*, **24**: 561-565. 1941.
- (6) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. The Effect of Copper on Lipase Activity in Cheddar Cheese. *J. Dairy Sci.*, **25**: 923-924. 1942.
- (7) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. Agitation and Temperature of Cheese Milk and the Development of Rancid and Unelean Flavors in Cheddar Cheese. *J. Dairy Sci.*, **26**: 1111-1119. 1943.
- (8) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. Further Studies on the Effect of Agitation and Temperature as a Cause of Rancidity in Cheese. *Can. Dairy Ice Cream J.*, **23** (3): 26-28, 60. 1944.
- (9) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. The Degree of Fat Dispersion in Cheese Milk and Its Relation to the Mechanism of Increased Lipase Action in Agitated Milk. *J. Dairy Sci.*, **28**: 79-83. 1945.
- (10) HOOD, E. G., HLYNKA, I., AND GIBSON, C. A. The Problem of Rancidity in Cheddar Cheese. *Can. Dairy Ice Cream J.*, **20** (3): 26. 1941.
- (11) LANE, C. B., AND HAMMER, B. W. Studies on the Fat of Cheddar Cheese. *Iowa State Coll. J. Sci.*, **13**: 149-155. 1939.

- (12) LANE, C. B., AND HAMMER, B. W. Relation of Fat Hydrolysis to Flavor Development in Cheddar Cheese. *J. Dairy Sci.*, 22: 432-433. 1939.
- (13) LANE, C. B., AND HAMMER, B. W. Effect of Lipolytic Enzymes on the Ripening of Cheddar Cheese. *J. Dairy Sci.*, 23: 519. 1940.
- (14) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. VI. Relationship of Fat Hydrolysis to the Ripening of Cheddar Cheese. *Iowa State Agr. Expt. Sta. Bull.* 291: 349-384. 1941.
- (15) PETERSON, M. H., AND JOHNSON, M. J. Learning Behavior of Enzymes in Ripening Cheese. *Ann. Rept. of the Director, Wis. Agr. Expt. Sta. Bull.* 461: Part 1, 39-40. 1943.
- (16) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Milk Lipase. *J. Dairy Sci.*, 26: 233-240. 1943.
- (17) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Cheese Lipase. *J. Dairy Sci.*, 31(1): 31-38. 1948.
- (18) RICE, F. E., AND MARKLEY, A. L. Proof of the Presence of Lipase in Milk and a New Method for the Detection of the Enzyme. *J. Dairy Sci.*, 5: 64-82. 1922.
- (19) WOLF, J. A Note on the Lipase of Some Lactic Acid Organisms. *Proc. Soc. Agr. Bacteriologists (Abs.)*. 1941.

## DETERMINATION OF CHEESE PROTEINASE<sup>1</sup>

MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE

*Departments of Biochemistry and Dairy Industry, College of Agriculture,  
University of Wisconsin, Madison*

During the past several years one of the major problems to be dealt with in the dairy industry has been concerned with the differences in ripening of raw milk and pasteurized milk Cheddar cheese. In an effort to attack this problem from the enzymatic viewpoint, it was necessary, first, to demonstrate the presence of hydrolytic enzymes (both lipases and proteinases) in the cheese, and, secondly, to devise methods suitable for the quantitative determination of these enzymes. This paper deals only with the proteolytic system of cheese. Lipases in cheese have been dealt with in other publications (2, 3).

The presence of proteolytic enzymes in Cheddar cheese has been generally assumed, and adequate evidence of their presence therein is contained in this report.

### DETERMINATION OF CHEESE PROTEINASE

*Substrate used and its preparation.* Casein was chosen as the substrate to be used in the method as finally adopted, not only because it is reproducible but also because it is very similar to the cheese curd itself.

The substrate is prepared as follows: Ten grams of purified casein (Labeo Brand, Casein Company of America) are weighed out with an accuracy of  $\pm 0.1$  g. To the weighed sample in a Waring Blendor or other suitable mixer, 25 ml. of distilled water and 25 ml. of 1 N sodium hydroxide are added. The power mixer is run at low speed for 2 minutes and then at high speed for 5 minutes. At the end of this time, while the mixer is still running, 40 ml. of a 0.2 molar sodium citrate solution are added and the mixer is allowed to run at high speed for 2 minutes longer. The substrate solution then is diluted to an approximate volume of 300 ml. At this point, while the mixer is running at high speed, 2 g. of gum ghatti dissolved in 30–40 ml. of distilled water are added. The total volume of the mixture should be less than 350 ml. The mixer is allowed to run for 5 minutes after the addition of the gum ghatti. A few drops of methyl red now are added. While the mixer is running, concentrated sulfuric acid is added dropwise until a pH of 5 is reached (red to methyl red). After a pH of 5 is reached, the mixer is allowed to run at high speed for 5 more minutes. At the end of this period the substrate preparation is quantitatively re-

Received for publication September 22, 1947.

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Cheese Institute.



moved from the mixer and diluted to 400 ml. The suspension obtained is run through a small hand homogenizer three to four times. After homogenization, the suspension is filtered through coarse qualitative filter paper to remove extraneous material and then is ready for use. Since substrate adequate for several enzyme analyses is prepared in the preceding operation, enough toluol to saturate the mixture is added as a preservative. Stock substrate solutions should be rehomogenized and filtered each day before use.

*Preparation and analysis of cheese samples for proteinase content.*

Twenty grams of the cheese are weighed out with an accuracy of  $\pm 0.1$  g. To the weighed sample in a Waring Blendor or other suitable mixer, enough distilled water is added to make an approximate volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set at high speed and allowed to run 7 minutes more. At the end of this time the mixture is in the form of a smooth, white cream. This mixture is quantitatively transferred to a 250-ml. graduate with less than 100 ml. of distilled water. The total volume of the mixture then is made to exactly 200 ml. in the graduate. After thorough mixing in the graduate, the suspension is homogenized five to six times through a small hand homogenizer. The cheese sample now is ready to be subjected to enzyme analysis.

In this method for the estimation of proteinase content of Cheddar cheese, a standard suspension of casein is digested under standard conditions, and the undigested casein is precipitated with trichloroacetic acid. The amount of unprecipitated protein split products, which is a measure of the amount of proteinase present, is estimated by measurement of the nitrogen contained therein. The nitrogen content of these unprecipitated protein split products, to be referred to hereafter as non-protein nitrogen, is determined by a colorimetric micromethod (1).

The enzyme analysis as finally adopted proceeds as follows: To 5 ml. of substrate suspension, 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of freshly prepared 0.015 molar cysteine hydrochloride, and 0.2 ml. toluol are added, and the whole is shaken vigorously for 2 minutes. This suspension and the homogenized cheese sample are placed separately in a water bath held at 40° C. When both have attained this temperature, from 0.5 to 3 ml. (depending upon the activity) of the cheese suspension are added to the substrate suspension, and the total volume is made to 10.2 ml. with distilled water. This digestion mixture is shaken vigorously for 30 seconds and then run quickly two to three times through a small hand homogenizer into a small test tube. After the removal of a 1-ml. aliquot for the determination of initial non-protein nitrogen present, the digestion mixture is incubated in a stoppered tube in the water bath at 40° C. for 5 hours. At the end of this time the digestion mixture is rehomogenized as before, and another 1-ml. aliquot is removed for analysis. The difference in non-protein nitrogen

content of the two aliquots represents casein hydrolysis and is a measure of proteinase present.

The analysis of the 1-ml. aliquots for non-protein nitrogen content is carried out as follows: The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 25 ml. of 0.3 N trichloroacetic acid in a large test tube. The tube is shaken vigorously and the contents filtered through a dry filter paper. An 11-cm. filter paper, such as Whatman no. 2, which does not adsorb protein split products, must be used. A suitable aliquot of the filtrate obtained is analyzed for nitrogen content by the colorimetric micromethod (1). The following alternative procedure for nitrogen analysis of the 1-ml. digestion mixture aliquots is used when the cheese samples are low in proteinase content. The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 4 ml. of 1 N trichloroacetic acid in a small test tube. The tube is shaken vigorously, and the contents are filtered through a dry 3-cm. filter paper of the same type as described above. The filtrate obtained is analyzed as above for nitrogen content. It usually is best to run a zero time analysis for each tube in both the above procedures since the non-protein nitrogen blank varies from time to time.

One cheese proteinase unit is defined as the amount of enzyme which, when diluted to 10.2 ml. and incubated for 5 hours under the above conditions, will liberate 1  $\mu$ g. of non-protein nitrogen. For instance, if 2 ml. of enzyme sample are used per 10.2 ml. of digestion mixture, and 1 ml. of digestion mixture (1/10.2 of the total) is added to 25 ml. of trichloroacetic acid (total volume of 26 ml.), and the observed increase in non-protein nitrogen is 7.5  $\mu$ g. per 5 ml. of trichloroacetic acid filtrate (5/26 of the total volume), the number of proteinase units present in the 2-ml. enzyme sample is  $7.5 \times 10.2 \times 26 \div 5$  or 397.8 units, or 198.9 proteinase units per ml. of enzyme sample. That the relation between amount of enzyme and increase in soluble nitrogen is linear is demonstrated by the experiments summarized in figure 3 and table 2. In figure 3 the relationship between quantity of cheese proteinase and amount of casein hydrolysis is presented. An arbitrary enzyme preparation was used in this experiment. As may be seen, care should be taken that the amount of enzyme preparation used is such that non-protein nitrogen increases of 2.75  $\mu$ g. or less per ml. of trichloroacetic acid filtrates are obtained. Above this level the casein hydrolysis obtained is not directly proportional to the quantity of proteinase present.

#### FACTORS AFFECTING THE ACTIVITY OF CHEESE PROTEINASE

*Effect of pH on cheese proteinase activity.* In figure 1 the pH activity curve for the hydrolysis of cheese proteinase is given. Determinations of enzyme activity at various pH values were carried out according to the procedure outlined previously in this paper. By means of a composite 0.5 molar

acetate, 0.5 molar phosphate, 0.5 molar borate buffer, pH was controlled throughout the pH range desired. Aliquots of this composite buffer were adjusted with 5 N sulfuric acid or 5 N sodium hydroxide to the pH values shown in figure 1. Proteinase determinations at these pH values were then carried out using in place of 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of the pH adjusted aliquots of the composite buffer.

It will be seen from the figure that casein is split most rapidly at pH 5. There also is a secondary optimum at pH 7 to 8. Other experiments have shown that this secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the proteolytic activity, however, seems to be quite constant throughout the cheese ripening period. The

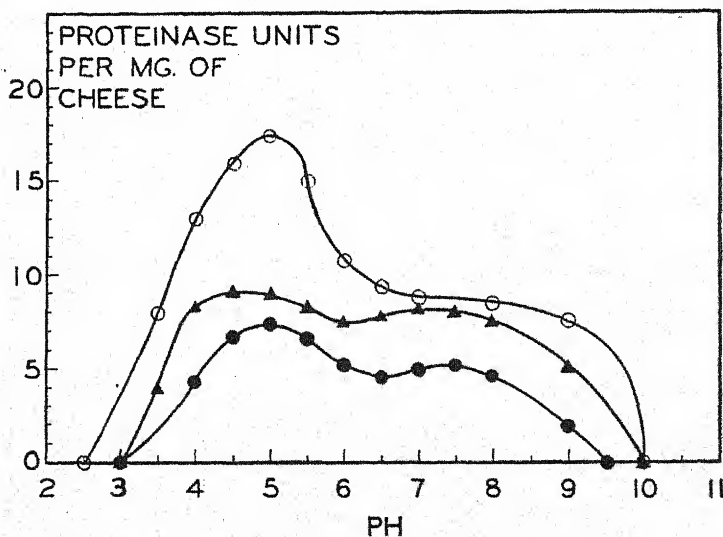


FIG. 1. Effect of pH on rate of casein hydrolysis by cheese extracts. Circles represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 6-month-old raw milk cheese A-100; triangles represent enzyme analyses without cysteine of extracts of 6-month-old raw milk cheese A-100; and dots represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 2-month-old raw milk cheese A-100.

presence of two peaks in the pH optimum of cheese proteinase indicates strongly that two or more proteinases are present. This also is indicated by cysteine activation of the enzymes at various pH values (fig. 1). The proteolytic activity at pH 5 is greatly activated by cysteine and probably represents endocellular bacterial proteinases liberated by bacterial autolysis (4). The proteolytic activity at pH 7 and above is unaffected by cysteine and probably represents extracellular proteinases liberated by bacteria during their normal life cycle. Since the pH of ripening cheese remains at or very near 5, only the proteolytic activity at pH 5 present in the cheese is important.



TABLE 1

*The effects of activators and buffers on cheese proteinase activity*

Substance	Concentration	Apparent cheese proteinase in sample
	M	(units/mg. cheese)
None .....	Activators	25.9
Potassium cyanide .....	0.01	64.5
Sodium sulfide .....	0.01	35.2
Cysteine hydrochloride .....	0.01	70.8
	Buffers*	
Aniline .....	0.04	29.2†
Pyridine .....	0.1	21.4
Phosphate .....	0.1	29.2
Borate .....	0.1	29.3
Phthalate .....	0.1	27.3
Acetate .....	0.1	29.4

\* All buffers were adjusted to pH 5 with 5 N sulfuric acid or 5 N sodium hydroxide prior to proteinase determination.

† The proteinase solution used in the buffer experiments was not the same as that used in the activator experiments.

*Effect of buffers on cheese proteinase activity.* In the development of a method for the estimation of cheese proteinase, it was necessary to find a buffer which did not affect the proteinase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese proteinase. The acetate buffer appears to meet the requirements and also is convenient.

*Effect of activators on cheese proteinase activity.* Various reducing agents known to possess definite activating properties for proteinases such as papain were tested for activating effect on cheese proteinase. As may

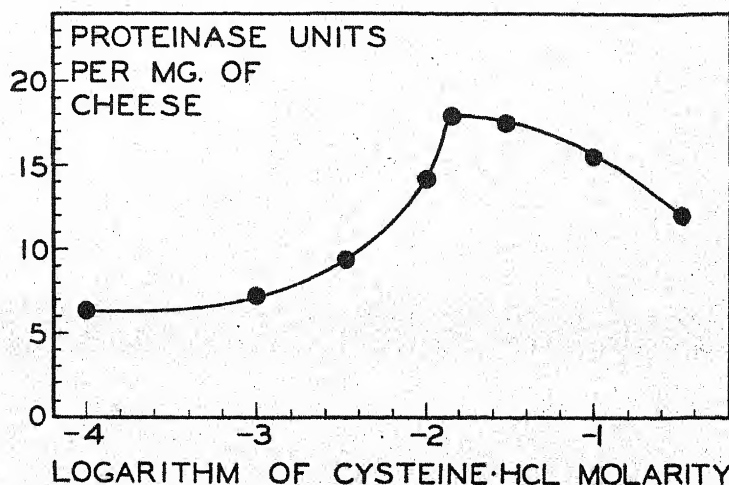


FIG. 2. Effect of cysteine hydrochloride concentration on cheese proteinase activity.

be seen from table 1, activation of the enzyme was obtained in all cases. Cysteine, however, gave more complete activation than sodium sulfide or potassium cyanide.

In order to determine the concentration of cysteine necessary to give maximum activation of cheese proteinase at pH 5, an experiment was run determining the proteinase activity of an enzyme preparation at various levels of cysteine hydrochloride. The results are given in figure 2. It may be seen that maximum activation of cheese proteinase occurred when the

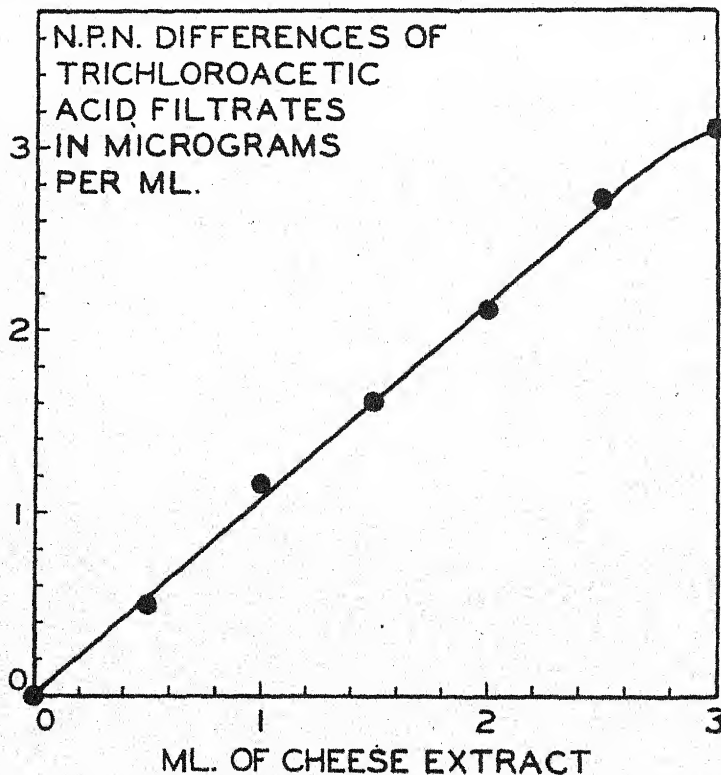


FIG. 3. Relation between quantity of cheese proteinase and amount of casein hydrolysis.

cysteine was present at a level of 0.015 molar. The use of cysteine hydrochloride in order to insure maximum activity of enzyme preparations at pH 5 then was incorporated into the method as outlined under "Preparation and analysis of cheese samples for proteinase content".

*Kinetics of cheese proteinase.* It already has been shown that the casein hydrolysis obtained is directly proportional to the quantity of cheese proteinase present (Fig. 3). In figure 4 the effect of incubation time on casein hydrolysis by cheese proteinase is presented. It may be seen that the hydrolysis of casein by cheese proteinase at pH 5 is linear up to the time when

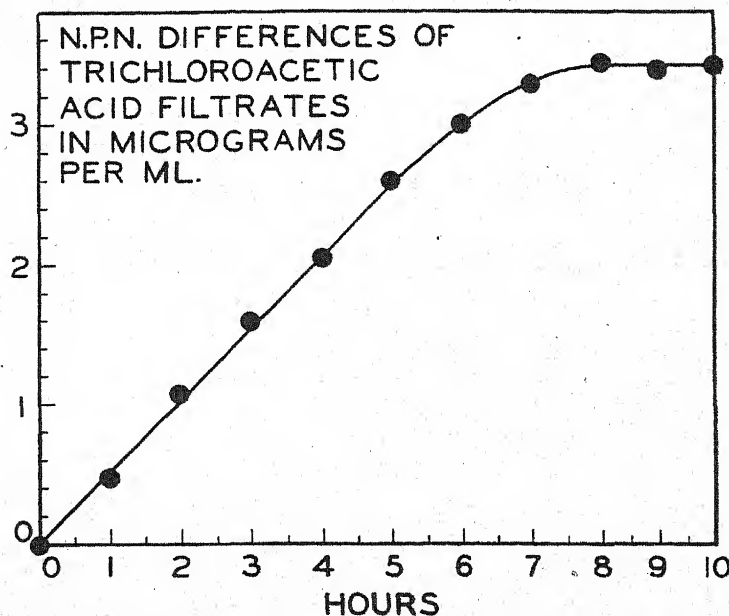


FIG. 4. Effect of incubation time on casein hydrolysis by cheese proteinase.

the increase of protein split products in the trichloroacetic acid filtrate is equivalent to 2.60  $\mu$ g. non-protein nitrogen per ml.

*Reliability of the method.* The reliability of the method was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 2. As may be seen, determinations run at various sample levels agree within 10 per cent.

#### SUMMARY

1. Data are presented showing the presence of proteolytic activity in Cheddar cheese.

2. A method is presented for the quantitative determination of proteo-

TABLE 2

*Effect of sample size on apparent cheese proteinase content of a cheese extract*

Sample size	Non-protein nitrogen increases in trichloro- acetic acid filtrates	Proteinase content
(ml.)	( $\mu$ g./ml.)	(units/ml.)
1.0	0.72	191
1.0	0.79	210
2.0	1.57	208
2.0	1.52	202
3.0	2.36	209
3.0	2.30	203



lytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

3. Hydrolysis of casein by the Cheddar cheese proteinase system is most rapid at pH 5. A secondary optimum occurs at pH 7 to 8.

4. Proteolytic activity of cheese suspensions at pH 5 is enhanced in the presence of reducing agents.

#### REFERENCES

- (1) JOHNSON, M. J. Isolation and Properties of a Pure Yeast Polypeptidase. *J. Biol. Chem.*, 137: 575-586. 1941.
- (2) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Cheese Lipase. *J. Dairy Sci.*, 31(1): 31-38. 1948.
- (3) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Lipase Activity during Making and Ripening of Cheddar Cheese. *J. Dairy Sci.*, 31(1): 39-46. 1948.
- (4) TARNANEN, J. Proteolytic Enzymes of Lactic Acid Bacteria. *Lab. Butterexportges. Valio m. b. H. Helsinki*, 1-79. 1930.

## PROTEINASE CONTENT OF CHEDDAR CHEESE DURING MAKING AND RIPENING<sup>1</sup>

MERLIN H. PETERSON, MARVIN J. JOHNSON, AND WALTER V. PRICE

*Departments of Biochemistry and Dairy Industry, College of Agriculture,  
University of Wisconsin, Madison*

It is a well recognized fact that proteinases play an important part in the ripening of Cheddar cheese because of their action on the cheese curd. Possible sources of cheese proteinases are the proteinases of milk, of rennet, and of microorganisms. It was first suggested by Babcock and Russell (1) and Babcock *et al.* (5) that an inherent milk proteinase, termed galactase, probably is the most important proteolytic agent in cheese ripening. Later work on variation of rennet levels in cheesemaking caused Babcock *et al.* (3) to revise their early views. Their final conclusion was that rennet is the most important proteinase source in cheese, its protein digestive action being due to the action of pepsin present in the rennet as an impurity. Sherwood (12), however, reported that the use of pepsin for rennet in Cheddar cheese resulted in 40-50 per cent less protein degradation than took place in normal rennet control cheeses. The ratios between amounts of different protein split products were quite similar for both types of cheese, indicating that nitrogen partition was identical. Sherwood (11) also found that although the extent of protein degradation in chloroform-treated Cheddar cheese was considerably less than for normal Cheddar cheese controls, the general course of nitrogen partition was the same for both chloroform-treated and normal cheese. The same work showed that normal Cheddar cheese had consistently higher levels of subpeptone nitrogen than chloroform-treated cheese. From these data Sherwood concluded that rennet itself is the most important proteolytic agent in Cheddar cheese ripening, and since its action extends but little beyond the peptone stage, any further proteolytic degradation must be due to bacterial action.

The purpose of the present investigation is to establish the relative importance of various sources of cheese proteinases, and also to present studies of the differences in proteolytic activity during the making and ripening of raw and pasteurized Cheddar cheese made from the same milk.

### METHODS

*Cheese proteinase.* The method used to determine proteolytic activity in Cheddar cheese reported by the present authors (10) was used in all cheese proteinase analyses. All analyses were made both with and without 0.015

Received for publication September 22, 1947.

<sup>1</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Cheese Institute.

molar cysteine in order to differentiate between cysteine-activated proteinases and those unaffected by cysteine.

*Cheesemaking procedure.* The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. A 920-lb. lot of the milk was divided into two equal portions. The first portion received no treatment. The second portion was subjected to pasteurization (holder method—30 min. at 145° F.). Each portion, after treatment, was placed in a separate vat, and both received identical subsequent treatment. Cheeses were made by an experienced maker according to standard procedures. From each vat two 22-lb. cheeses were obtained. The green cheeses produced were kept at 68° F. for a period of 2-3 days and then were transferred to a cold room (40-45° F.) for the remainder of the ripening period.

*Sampling.* Samples for enzyme analysis were taken at various intervals during the making process and ripening period. Sampling times used are indicated in the figures and tables.

#### PROTEOLYTIC ACTIVITY OF CHEDDAR CHEESE DURING MAKING AND RIPENING

*Proteinase content of raw and pasteurized Cheddar cheese.* In figures 1-a, 1-b, 2-a, and 2-b the averages of the proteolytic activities at pH 5 of a series of pairs of pasteurized milk cheeses and raw milk cheeses made by the procedure described under "Methods" are given. Figures 1-b and 2-b cover the entire ripening period. Figures 1-a and 2-a show, on an enlarged scale, the changes occurring during the making and the first few days of ripening.

Figures 1-a and 1-b, representing the proteolytic activity in raw milk Cheddar cheese, will be considered first. In figure 1-a, it will be noted that a small amount of proteolytic activity is present in the milk at the time it is placed in the vat. This represents milk proteinase, the significance of which will be discussed later. The first important increase in proteolytic activity occurs 90 to 120 minutes after the start of making. While part of this increase can be attributed to proteinases of the added rennet, comparison of figures 1-a and 2-a indicates that most of this increase probably is due to bacteria growing and producing extracellular proteinases during the making process. Since the corresponding increase in the proteolytic activity of pasteurized milk cheese (fig. 2-a) is much smaller, it is believed that the bacteria responsible are destroyed in the pasteurization process. No further change in proteolytic activity occurs until after the cheese is in its final form and has been stored. In the first few days of ripening a large increase in cysteine-activated proteinases occurs. The use of cysteine here is valuable in that it affords a means of differentiation in the type of proteinases present in the cheese and thus affords a clue to the source of these



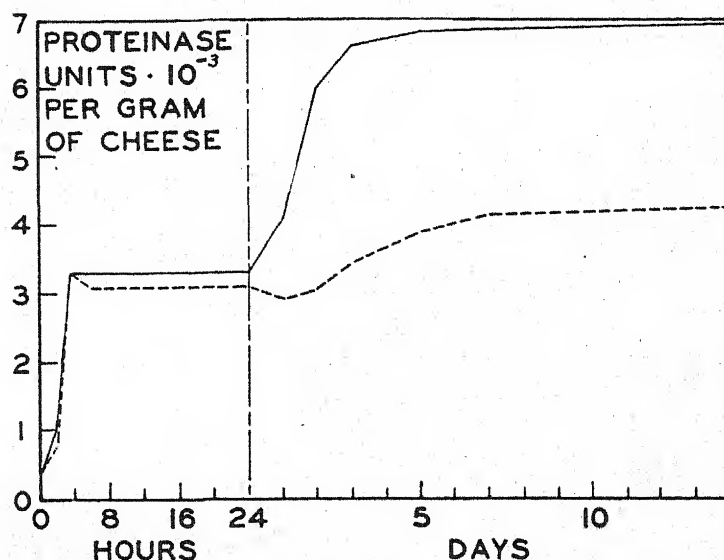


FIG. 1-a. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

proteinases. It should be emphasized that in the ripening cheese where strictly anaerobic conditions are present (6), the cysteine-activated proteinases undoubtedly are present in a fully active state.

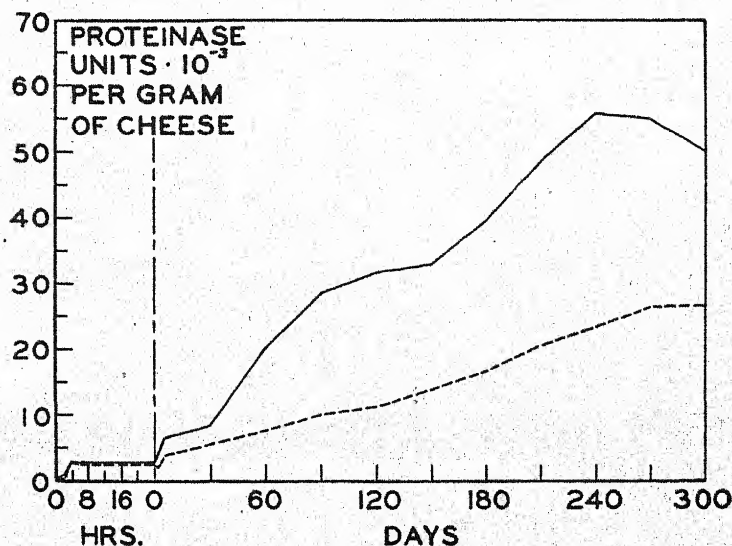


FIG. 1-b. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and a 300-day ripening period; same cheeses as Fig. 1-a. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

Figures 1-*a* and 1-*b* show that the amount of proteinase in the milk is very small compared to the proteinase of the cheese. Consequently, even if the milk proteinase is carried over quantitatively into the cheese, its role in ripening must be small. It also will be noted that the proteolytic activity at pH 5 of the added rennet is relatively small; it is readily seen, therefore, why such large amounts of rennet extract must be added to Cheddar cheese in order to increase markedly the rate of ripening (2, 3, 4, 7, 8, 9, 14).

Figure 1-*a* indicates that most of the proteinase of the young cheese is bacterial in origin. Part of this is produced in the vat, and the remainder is set free during the first few days of ripening. Figure 1-*b* shows that as

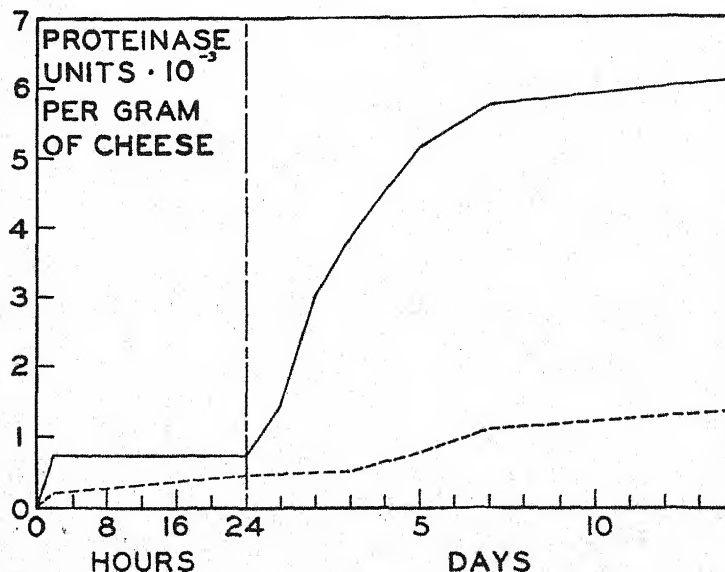


FIG. 2-*a*. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

the cheese ripens its proteinase content increases greatly. It is very apparent that only a small portion of the total proteolytic activity is contributed by the milk and the rennet when standard Cheddar cheesemaking procedures are employed. The greater portion of the proteinase increase is cysteine-activated and may represent endocellular bacterial proteinases liberated by bacterial autolysis (13).

The proteinase activity of pasteurized milk cheese at various ages is shown in figures 2-*a* and 2-*b*. In pasteurized milk cheese (fig. 2-*a*) there is no increase in proteinase in the vat other than that introduced with the rennet. There is a relatively large increase in cysteine-activated proteinase during the first few days of ripening. Figure 2-*b* shows that the total proteinase content of pasteurized milk cheese during ripening is less than that

of raw milk cheese, and that in pasteurized milk cheese much less cysteine-activated proteinase is present.

Since the sources of the proteinases found in Cheddar cheese during the later ripening period must be in large part bacterial, it might readily be assumed that some of the differences between raw and pasteurized milk cheese made from the same lot of milk could be attributed to this difference in cysteine-activated proteinase content. The bacteria responsible probably are species which are greatly reduced in number during pasteurization. It also may be seen from figures 1-b and 2-b that the content of proteinase, not activated by cysteine, becomes greater in the pasteurized milk cheese than

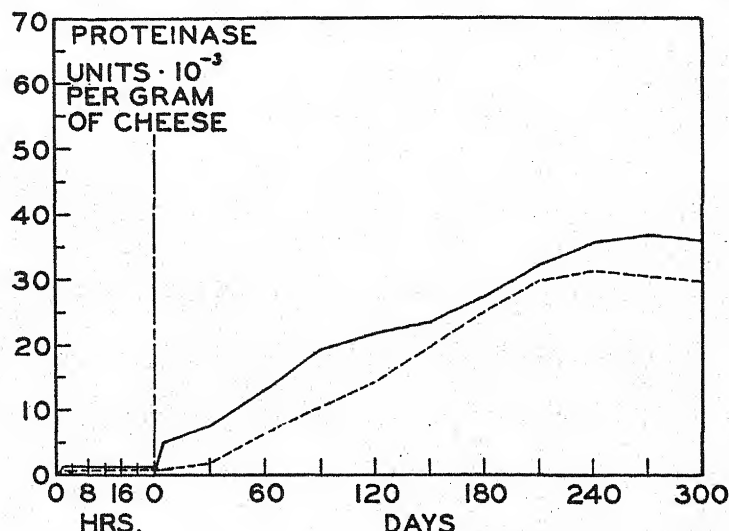


Fig. 2-b. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 2-a. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

that of the raw milk cheese after 80 days of ripening and remains greater throughout the rest of the ripening period.

During ripening, flavor development always is more complete in raw milk cheese than in pasteurized milk cheese of the same age. Since the difference in proteinase content between the two types is largely in the cysteine-activated fraction, it follows that cysteine-activated proteinases may be responsible, in part, for the more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

*Reproducibility of cheeses.* The data of figures 1 and 2 represent aver-



Figures 1-*a* and 1-*b* show that the amount of proteinase in the milk is very small compared to the proteinase of the cheese. Consequently, even if the milk proteinase is carried over quantitatively into the cheese, its role in ripening must be small. It also will be noted that the proteolytic activity at pH 5 of the added rennet is relatively small; it is readily seen, therefore, why such large amounts of rennet extract must be added to Cheddar cheese in order to increase markedly the rate of ripening (2, 3, 4, 7, 8, 9, 14).

Figure 1-*a* indicates that most of the proteinase of the young cheese is bacterial in origin. Part of this is produced in the vat, and the remainder is set free during the first few days of ripening. Figure 1-*b* shows that as

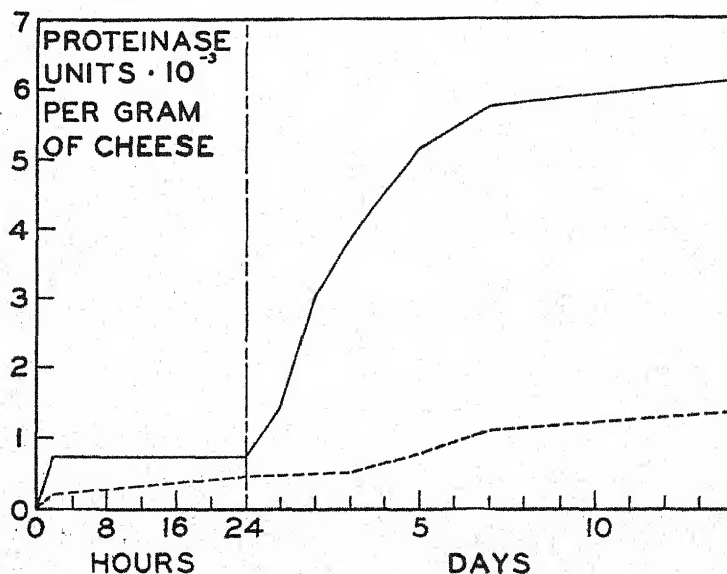


FIG. 2-*a*. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

the cheese ripens its proteinase content increases greatly. It is very apparent that only a small portion of the total proteolytic activity is contributed by the milk and the rennet when standard Cheddar cheesemaking procedures are employed. The greater portion of the proteinase increase is cysteine-activated and may represent endocellular bacterial proteinases liberated by bacterial autolysis (13).

The proteinase activity of pasteurized milk cheese at various ages is shown in figures 2-*a* and 2-*b*. In pasteurized milk cheese (fig. 2-*a*) there is no increase in proteinase in the vat other than that introduced with the rennet. There is a relatively large increase in cysteine-activated proteinase during the first few days of ripening. Figure 2-*b* shows that the total proteinase content of pasteurized milk cheese during ripening is less than that

of raw milk cheese, and that in pasteurized milk cheese much less cysteine-activated proteinase is present.

Since the sources of the proteinases found in Cheddar cheese during the later ripening period must be in large part bacterial, it might readily be assumed that some of the differences between raw and pasteurized milk cheese made from the same lot of milk could be attributed to this difference in cysteine-activated proteinase content. The bacteria responsible probably are species which are greatly reduced in number during pasteurization. It also may be seen from figures 1-b and 2-b that the content of proteinase, not activated by cysteine, becomes greater in the pasteurized milk cheese than

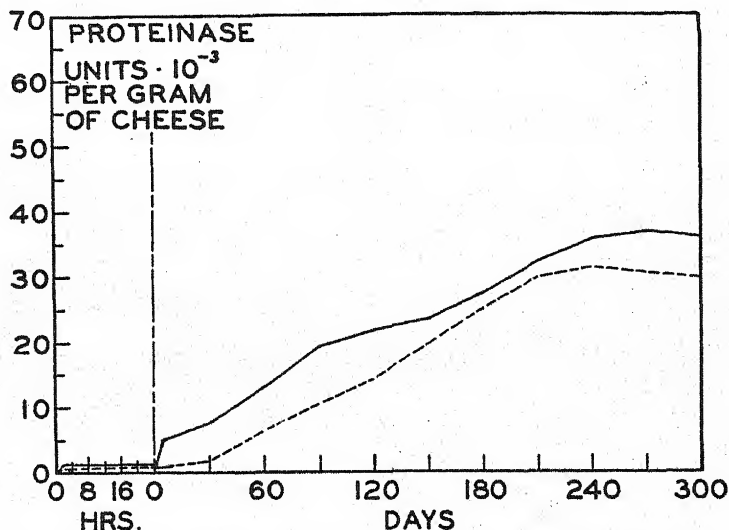


FIG. 2-b. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 2-a. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

that of the raw milk cheese after 80 days of ripening and remains greater throughout the rest of the ripening period.

During ripening, flavor development always is more complete in raw milk cheese than in pasteurized milk cheese of the same age. Since the difference in proteinase content between the two types is largely in the cysteine-activated fraction, it follows that cysteine-activated proteinases may be responsible, in part, for the more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

*Reproducibility of cheeses.* The data of figures 1 and 2 represent aver-

TABLE 1  
*Proteinase content of Cheddar cheese during ripening*

Cheese no.*	Proteinase content in units per milligram of cheese									
	With cysteine†					Without cysteine				
	0 days	10 days	30 days	90 days	180 days	0 days	10 days	30 days	90 days	180 days
1015R	3.5	6.6	8.2	27.5	36.6	2.6	4.1	5.1	9.5	16.3
1022R	3.0	6.2	7.5	21.3	33.1	2.1	3.8	4.8	9.2	15.8
1103R	3.9	7.0	8.3	30.1	42.7	2.5	4.3	5.0	9.8	17.0
1119R	4.1	7.0	8.4	31.2	41.7	2.8	4.5	5.3	10.0	17.4
1124R	3.6	6.7	8.1	29.0	41.3	2.6	3.9	5.1	9.6	16.5
1201R	3.5	6.4	8.0	28.0	34.8	2.4	3.7	5.0	9.6	15.2
1015P	0	5.5	7.2	18.0	25.8	0	1.1	1.9	9.3	27.2
1022P	0	4.9	7.7	17.5	22.1	0	0.8	1.4	10.0	26.5
1103P	0	5.7	7.6	18.9	29.1	0	1.2	1.5	10.1	28.1
1119P	0	6.0	7.9	17.4	28.5	0	1.5	2.1	10.4	30.1
1124P	0	5.6	7.0	17.7	24.9	0	1.0	1.6	8.9	26.2
1201P	0	5.4	7.2	19.9	22.9	0	1.0	1.0	8.7	24.7

\* The letter R indicates a raw milk cheese, P a pasteurized milk cheese. Cheeses made from the same lot of milk have the same number.

† Proteinase determinations were made at pH 5 both with and without 0.015 M cysteine (10).



ages of six pairs of raw and pasteurized milk cheeses. Analyses after 180 days, however, were made on only one pair of cheeses. The individual curves for the six pairs of cheeses were very similar. The individual analyses are presented in table 1 and may be seen to check closely.

#### SUMMARY

1. The proteinase content of a series of pairs of raw and pasteurized milk Cheddar cheeses has been determined at intervals during the making and ripening periods.
2. The active proteinase in ripening Cheddar cheese is largely of bacterial origin; only a small fraction of the total activity is contributed by the milk and the rennet.
3. Pasteurized milk Cheddar cheese is characterized by a lower content of cysteine-activated proteinase than raw milk Cheddar cheese.

#### REFERENCES

- (1) BABCOCK, S. M., AND RUSSELL, H. L. Unorganized Ferments of Milk: A New Factor in the Ripening of Cheese. Wis. Agr. Expt. Sta. 14th Ann. Rept., 161-193. 1896.
- (2) BABCOCK, S. M., AND RUSSELL, H. L. Curing of Cheddar Cheese with Especial Reference to Cold-Curing. Wis. Agr. Expt. Sta. Bull. 94. 1902.
- (3) BABCOCK, S. M., RUSSELL, H. L., AND VIVIAN, A. Influence of Rennet on Cheese-Ripening. Wis. Agr. Expt. Sta. 17th Ann. Rept., 102-122. 1906.
- (4) BABCOCK, S. M., RUSSELL, H. L., VIVIAN, A., AND BAER, U. S. Influence of Varying Quantities of Rennet on Cold-Cured Cheese. Wis. Agr. Expt. Sta. 19th Ann. Rept., 174-179. 1902.
- (5) BABCOCK, S. M., RUSSELL, H. L., VIVIAN, A., AND HASTINGS, E. G. The Action of Proteolytic Ferments on Milk with Special Reference to Galactase, the Cheese-Ripening Enzyme. Wis. Agr. Expt. Sta. 16th Ann. Rept., 157-174. 1898.
- (6) DAVIS, J. G. Studies in Cheddar Cheese. I. The Oxidation-Reduction Potentials of Ripening Cheddar Cheese. J. Dairy Research, 3: 241-253. 1932.
- (7) DOANE, C. F. Investigations in the Manufacture and Curing of Cheese. VI. The Cold-Curing of American Cheese. U. S. Dept. Agr. Bur. Animal Ind. Bull. 85. 1906.
- (8) N. Y. (GENEVA) AGR. EXPT. STA. Experiments in the Manufacture of Cheese. II. Study of Cheese Ripening Process. N. Y. (Geneva) Agr. Expt. Sta. Bull. 54 (New Series): 261-269. 1893.
- (9) ORLA-JENSEN, P. Studien uber die Enzyme in Kase. Landw. Jahr. Schweiz, 14: 197-233. 1900.
- (10) PETERSON, W. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Cheese Proteinase. J. Dairy Sci., 31(1): 47-54. 1948.
- (11) SHERWOOD, I. R. The Role of Rennet in the Ripening of Cheddar Cheese. J. Dairy Research, 6: 204-217. 1935.
- (12) SHERWOOD, I. R. The Function of Pepsin and Rennet in the Ripening of Cheddar Cheese. J. Dairy Research, 6: 407-421. 1935.
- (13) TARNANEN, J. Proteolytic Enzymes of Lactic Acid Bacteria. Lab. Butterexportges. Valio m. b. H. Helsinki, 1-79. 1930.
- (14) VAN SLYKE, L. L., AND HART, E. B. Conditions Affecting Chemical Changes in Cheese Ripening. N. Y. (Geneva) Agr. Expt. Sta. Bull. 236: 131-163. 1903.



# THE HERITABILITY OF OFFICIAL TYPE RATINGS AND THE CORRELATION BETWEEN TYPE RATINGS AND BUTTER-FAT PRODUCTION OF AYRSHIRE COWS<sup>1</sup>

W. J. TYLER AND GEORGE HYATT, JR.

*Department of Dairy Husbandry, West Virginia University, Morgantown*

The selection of dairy cattle for breeding purposes usually is based on the type and the production records of the animals, their ancestors or their progeny. The consideration given to type in a selection program should be determined by its economic importance to the breeder—i.e., by the advertising value and the increase in selling prices of animals with high classification ratings, by the relationship between type and production, and by the heritability of type. The economic importance of type varies from herd to herd, depending on the sales policy used by the breeder. Neither the correlation between type and production nor the heritability of type should vary much from herd to herd within a breed.

The purpose of this study was to estimate the heritability of single type ratings and to compute the correlation between type and production in Ayrshire cattle.

## EXPERIMENTAL PROCEDURE

The data used in this study were the type ratings of Ayrshire cows classified between March, 1942, and May, 1946, by official inspectors in accordance with the rules of the classification program of the Ayrshire Breeders' Association. The participation of Ayrshire herds in this program is voluntary. According to the plan, the breeder submits for official inspection all of his cows that have freshened one or more times. These cows are given a single rating of one of five grades: Excellent, Very Good, Good Plus, Good, and Fair.

The rating and other information on each cow were coded and punched on International Business Machine cards. These cards were sorted according to inspector and date of classification. The correlation coefficients between ratings of related animals that were classified on the same day by the same inspector were computed and used to estimate the proportion of the total variability in ratings that is transmitted from parents to their offspring. In this paper this transmitted portion is called the heritability. From the correlation between the type ratings of paternal sisters and the regression of daughter's rating on dam's rating after removing the influence of the sires, the heritability of single type ratings was estimated.

The butterfat production records of classified cows were used to calculate

Received for publication October 2, 1947.

<sup>1</sup> Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 380.



the correlation between type and production. Type was paired with the first record, with the record begun nearest to the date of classification, and with the average of all records. All butterfat records were adjusted to a mature-equivalent, 305-day, twice-a-day milking basis.

## RESULTS

*Heritability of type.* The classification ratings of 3,738 cows sired by 368 bulls were used to compute the correlation between the ratings of paternal sisters. Only those sires with six or more daughters that were classified the same day were included in this study. This procedure should have eliminated most of those sires which would be represented by a few highly selected mature daughters. The frequency distribution of the ratings of these 3,738 cows and the distribution of 8,573 Ayrshire cows that were classified during the same period are given in table 1. The agreement between the two columns showing percentages indicates that the group of 3,738 cows is a representative sample of the breed.

TABLE 1

*Frequency distribution of the ratings for paternal sisters and for the Ayrshire breed*

Rating	No. of paternal sisters	Percentage of total	Breed percentage of total*
Excellent .....	109	2.9	3.7
Very good .....	831	22.2	22.1
Good plus .....	1627	43.6	42.7
Good .....	987	26.4	26.8
Fair .....	184	4.9	4.7
Total .....	3738	100.0	100.0

\* This is the distribution of the classification ratings of 8,573 Ayrshire females classified between March, 1942, and May, 1946.

The variance of these type ratings was divided into variation between inspectors, between sires within inspectors, and between daughters by the same sire, as shown in table 2 (9). The standard deviation of all ratings was 0.9. The variance component *A* represents variation between daughters by the same sire, while component *B* is the additional variance which can be ascribed to difference between sires, and *C* to differences between inspectors. The ratio  $\frac{B}{A+B}$  is the average correlation between daughters by the same sire. In these data this correlation was 0.12 with a standard error of 0.04.

There were 1,601 cows whose dams were classified on the same day by the same inspector. These cows were sired by 789 sires. The analysis of covariance technique was used (table 3) to obtain the regression of daughter's rating on dam's rating within sires and this coefficient was  $0.14 \pm 0.034$ . The regression of offspring on dam within sires avoids much

TABLE 2  
*Analysis of the variation in type ratings of 3,738 cows sired by 368 bulls*

Source of variation	Degrees of freedom	Mean square	Variance components of mean square*	Individual components
Between inspectors	9	15.330†	$A + 10.16$	$C = 0.037$
Between sires within insps.	358	1.634†	$B + 373.7$	$B = 0.095$
Within sires within insps.	3370	0.667	$A$	$A = 0.867$
Summary	3737	0.795	$A + 0.998$	$A + B + C = 0.799$
			$\frac{B}{A+B} = 0.12$	

\* Coefficients for the components of variance were computed by the method used by Dickerson (4).

†  $P < 0.01$ .

of the environmental contribution which would be found in a gross correlation or regression between parent and offspring.

Estimates of the heritability<sup>2</sup> of type in cattle can be made from the above relationship. In a random breeding population the correlation between traits of paternal sisters may be expected to contain 0.25 of the additive genetic variance, 0.06 or less of the epistatic variance, plus the environmental portion of the variance times the correlation between the environments of the half sisters (10). However, in those herds where the only source of new stock is through sires, the average genetic relationship between two animals by the same bull often is greater than 0.25. In most cases the animals used in this study were from herds using only one or two sires. Therefore, the average genetic relationship of the paternal sisters should be approximately 0.30. Since the environmental contribution to the

TABLE 3

*Analysis of covariance of type ratings of dams and daughters and the intra-sire regression of daughter's rating on dam's rating*

Source of variation	Degrees of freedom	Sums of squares		Cross products	Regression dau. on dam
		Daughters	Dams		
Between sires .....	788	719	830	240	.....
Within sires .....	812	452	481	66	0.14
Total .....	1600	1171	1311	306	.....

paternal sister correlation could not be determined accurately, the heritability of type ratings could be estimated to be somewhat less than  $\frac{1}{0.30} \times 0.12$  or 0.40 from these data.

The intra-sire regression coefficient of daughter's rating on dam's rating should contain 0.5 of the additive genetic variance plus about 0.25 of the epistatic variance. Non-random matings would not affect the intra-sire regression coefficient of daughter on dam (6). Hence, the estimate of heritability from these data would be about  $2 \times 0.14$  or 0.28.

When the two estimates of heritability are combined, the average figure is 0.30 and the 95 per cent fiducial limits are 0.19 and 0.42. This means that the chances are very good (19 out of 20) that the real heritability of type ratings lies within these limits.

*Correlation between type and production.* The official ratings, number of cows, percentage of total, and average butterfat production (first record, record started nearest to the date of classification, and the average of all records) of the 5,177 cows that were classified and also had production

<sup>2</sup> Lush defined the degree of heritability as "the fraction of the observed variance which was caused by differences in heredity" (6). This fraction may result from additive gene effects, interaction of allelic genes (dominance), and interaction of non-allelic genes (epistasis).



records are given in table 4. On the basis of the percentages for the classification groups for all classified Ayrshires (table 1), a larger percentage of the Excellent and Very Good cows have production records. This is similar to Copeland's findings (1, 2) in his study of classified Jersey cows, and means either that the herds on official test were culled more closely before the inspector classified the cattle, that these herds were inherently better in type conformation than the herds that were not on test, or that inspectors are influenced by the records (consciously or unconsciously) and thereby place higher producers in the Excellent and Very Good classes when they know about production and do not when the production is unknown to them.

The butterfat production of cows increased with each rise in type rating with the exception of the average of the first butterfat records of the Good and Fair groups of cows.

In order to remove the effect of herd on the correlation between type and

TABLE 4

*Frequency distribution of the ratings of the cows with production records and the average production of butterfat for each rating*

Classification rating	No. of cows	Percentage of total	Av. butterfat production		
			1st records	Nearest record	Av. all records
Excellent .....	320	6.2	415	417	412
Very good .....	1479	28.6	394	385	386
Good plus .....	2105	40.6	376	367	369
Good .....	1097	21.2	361	352	353
Fair .....	176	3.4	363	345	350
Summary .....	5177	100.0	380	371	372

butterfat production, the data were analyzed by the analysis of covariance to obtain the intra-herd correlation and regression of butterfat production on type rating. The correlation and regression coefficients of the first, nearest, and average of all butterfat records on type rating are given in table 5. The between-herd correlation coefficients,  $r = 0.53, 0.42$ , and  $0.50$ , are highly significant and mean that those herds classified as the best type tended to have the highest average production per cow. Within each herd, however, the correlation coefficients between type and butterfat production were lower,  $r = 0.16, 0.16$ , and  $0.19$ , but still statistically significant. The regression coefficients show that within a herd the butterfat production of the cows increased about 12-14 lb. for each increase in type grade. However, herds that averaged one classification grade higher in type also averaged about 82 lb. more butterfat per cow.

#### DISCUSSION

The objectives of this study in dairy cattle breeding were to determine the heritability (transmitted portion of the superiority of parents over

average of group from which parents were selected) of type and the correlation between type and production. On the basis of the studies of economic characteristics in swine (3), and milk and butterfat production in dairy cattle (8), it would seem that the results found here might well apply to other breeds of dairy cattle.

The results have shown that the heritability of single type ratings in Ayrshire cows probably lies between 0.19 and 0.42. This indicates that selection for type through a type classification program should improve the type of future generations of dairy cattle. If type was considered in the selection program, the breeder could expect the next group of offspring from the selected parents to be approximately the heritability times the difference between the type of the selected parents and the type of the group or herd from which the parents came. For example, if the average type of a herd was Good Plus (82.5) and the animals selected as parents averaged Very

TABLE 5

*Analysis of covariance and the correlation and regression coefficients of butterfat production on type rating*

Source of variation	Degrees of freedom	Correlation coefficients*			Regression coefficients*		
		1st record & type	Nearest record & type	Av. all records & type	1st record on type	Nearest record on type	Av. all records on type
Between herds .....	303	0.527	0.417	0.497	82.6	80.3	83.2
Within herds .....	4873	0.156	0.159	0.193	11.6	13.9	12.9
Summary .....	5176	0.193	0.184	0.220	15.7	17.7	16.8

\*  $P < 0.01$  for all values found.

Good (87.5), then the best estimate of the type of the offspring of these parents is  $82.5 + 0.2(87.5 - 82.5)$ , or 83.5 if the heritability of type is 0.2, and 84 and 84.5 if the heritability is either 0.3 or 0.4, respectively. Actually, the improvement in type would not be this fast unless the sire's breeding value for type was as equally well known as the type of the cows selected as parents. Estimates of a sire's breeding value for type could be made from a progeny test or from a pedigree study. The correlation between the type of a sire and the type of his daughters is not well known, although Copeland's figures indicated a low relationship (1).

The intra-herd relationship of type and butterfat production was 0.16 between type and single records, and 0.19 between type and average of all records. These correlation coefficients are very similar to those reported by Lush (7) for Holstein-Friesian cows, but somewhat smaller than the coefficients which Copeland (2) computed between type and butterfat records of Jersey cows. The difference between the correlation coefficient between type and a single butterfat record (first or nearest) and the correlation

coefficient between type and the average of all butterfat records is not statistically significant, but it is in the expected direction.<sup>3</sup> Intra-herd correlations were computed for this study because in most cases selection is practiced among animals that are raised on the same farm. Selection within herds for type only would not result in any substantial increase in butterfat production. Likewise, selection for butterfat alone would not increase the type of the animals to any great extent.

Hazel (5) has described a procedure to derive selection indexes. Before an index can be made for butterfat and type, however, the relative economic importance of each must be determined, and likewise the correlation between the genotypes (breeding value) for type and butterfat must be known. The advantage of an index is that improvement would be made in both type and production so that the gain would mean most to the breeder in dollars and cents. It seems probable that other characteristics (such as longevity and reproductive behavior) should be considered in deriving an index for selecting dairy cattle.

#### SUMMARY

The classification ratings of 3,738 paternal sisters and of 1,601 cows out of classified dams were used to estimate the portion of the differences in official type ratings that is transmitted from parents to offspring. This portion is called heritability and from these data this figure is estimated to be 0.3. Thus offspring inherit about one-third of the observed superiority of the parents' type, and herd improvement in the type of dairy cattle can be made by the selection of animals that are above the herd average in type for parents of the next generation.

The correlation between classification rating and production of butterfat was computed from data on 5,177 cows. Within herds there was an average increase of 13 lb. of butterfat for each increase of one grade of type. The relationships between the classification rating and the first butterfat record of a cow, between the type rating of a cow and her butterfat record begun nearest the date of classification, and between the type rating of a cow and the average of all her butterfat records were statistically highly significant, but they were so low that their practical significance is quite small. The correlation coefficients were 0.16, 0.16, and 0.19, respectively.

#### ACKNOWLEDGMENTS

The authors wish to thank Mr. C. T. Conklin, Secretary of the Ayrshire Breeders' Association, for making available the records punched on International Business Machine cards on which this study was based. They express their appreciation to Dr. W. W. Armentrout, Head, Agricultural

<sup>3</sup> The average number of records per cow was 3.5. If the repeatability of butterfat records is assumed to be 0.4, then the expected correlation between one type rating and the average of all butterfat records is  $0.16 \times 1.32$  or 0.21.



Economics Department, West Virginia University, and Miss Margaret Cross for the sorting and tabulation of the International Business Machine cards. They also are indebted to Dr. H. O. Henderson, Head, Department of Dairy Husbandry, West Virginia University, and Dr. A. B. Chapman, Department of Genetics, University of Wisconsin, for their valuable suggestions in the preparation of this manuscript.

## REFERENCES

- (1) COPELAND, L. The Old Story of Type and Production. *J. Dairy Sci.*, 21: 295-304. 1938.
- (2) COPELAND, L. The Relationship between Type and Production. *J. Dairy Sci.*, 24: 297-304. 1941.
- (3) CRAFT, W. A. Swine Breeding Research at the Regional Swine Breeding Laboratory. U. S. Dept. Agr. Misc. Pub. 523. 1943.
- (4) DICKERSON, G. E. Experimental Design for Testing Inbred Lines of Swine. *J. Animal Sci.*, 1: 326-341. 1942.
- (5) HAZEL, L. N. The Genetic Basis for Constructing Selection Indexes. *Genetics*, 28: 476-490. 1943.
- (6) LUSH, J. L. Intra-sire Correlations or Regressions of Offspring on Dam as a Method of Estimating Heritability of Characteristics. *Proc. Am. Soc. Animal Production*, 1940: 293-301. 1940.
- (7) LUSH, J. L. *Animal Breeding Plans*. 2nd Ed. Iowa State College Press, Ames, Iowa. 1943.
- (8) LUSH, J. L., AND STRAUS, F. S. The Heritability of Butterfat Production in Dairy Cattle. *J. Dairy Sci.*, 25: 975-982. 1942.
- (9) SNEDECOR, G. W. *Statistical Methods*. 4th Ed. Iowa State College Press, Ames, Iowa. 1946.
- (10) WRIGHT, S. The Analysis of Variance and the Correlation between Relatives with Respect to Deviation from an Optimum. *J. Genetics*, 30: 243-256. 1935.

## VARIATIONS IN TYPE RATINGS OF INDIVIDUAL AYRSHIRE COWS<sup>1</sup>

GEORGE HYATT, JR., AND W. J. TYLER

*Department of Dairy Husbandry, West Virginia University, Morgantown*

Type classification programs have been adopted by all the major dairy cattle breed associations. With the exception of the Guernsey breed, in order to participate in this program each breeder must submit all of the registered cows in his herd for classification. At a subsequent reclassification of the herd new ratings on previously classified cows will be official only if the new rating is higher than the previous official rating. Before a definite appraisal of the type classification program can be made, it is desirable to know as much as possible about the amount of variation that occurs when the same cows are classified several times throughout a lifetime. This investigation sought to determine: (a) The degree or amount of variation that occurs when the same cows are classified several times in their lifetime, (b) the degree of agreement between ratings given the same cow by the same inspector and by different inspectors at different times, (c) the possibility that age of animal, stage of lactation, and condition of animal influence the official type rating, (d) the value of photographic records in studying the type of animals from birth to maturity, and (e) the reasons why the ratings of some cows undergo large changes.

### EXPERIMENTAL PROCEDURE

The data for this investigation were collected from the Ayrshire herd (known as the Reymann Memorial Herd) at the West Virginia Agricultural Experiment Station. This herd represents approximately 25 years of a continuous, carefully controlled breeding project. No females have been brought into the herd in that period of time, and each normal female that is dropped in the herd must be raised and retained through at least one 305-day lactation before her disposal. The feeding and management of the herd has been kept as nearly constant as possible over the 25-year period. Each animal is bred to freshen each year, allowing 6 to 8 weeks for the dry period.

From 1942 through 1946 all the females that had calved were rated for type approximately three times yearly. During this period 138 cows were rated. Of this number, 32 were rated once, 26 twice, 17 three times, 8 four times, 6 five times, 9 six times, 12 seven times, 9 eight times, 5 nine times, 4 ten times, 2 eleven times, 3 twelve times, and 5 thirteen times. In

Received for publication October 2, 1947.

<sup>1</sup> Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 382.

TABLE 1  
Classification ratings of cows in the Reymann Memorial Herd rated three times or more from May 16, 1942, to December 20, 1946

Cow no.	Inspector and date													Range
	R 5-42	R 10-42	S 1-43	R 5-43	T 7-43	U 10-43	U 1-44	V 4-44	W 12-44	X 4-45	Y 12-45	T 5-46	Z 12-46	
462	3*	3	3	4	3	3	3	5	5	...	...	...	...	1
473	5	5	3	5	5	5	5	4	4	...	...	...	...	0
491	4	4	5	5	5	5	5	4	4	4	4	5	4	1
500	4	4	4	5	5	5	5	3	3	4	4	...	...	1
501	4	4	2	3	2	1	1	4	4	3	5	4	...	2
521	4	3	3	4	3	4	4	4	3	...	...	...	4	3
526	3	3	3	4	3	4	4	4	3	4	...	...	...	2
530	4	4	4	4	4	4	4	4	3	4	5	4	4	1
537	5	4	4	5	4	4	4	4	5	5	...	...	...	1
539	4	4	4	4	4	4	4	4	3	4	4	4	4	1
561	4	4	4	4	4	4	4	4	4	4	...	...	...	1
569	2	4	4	4	4	4	4	4	4	4	...	...	...	1
577	4	4	4	4	4	4	4	3	3	3	...	...	...	1
592	3	4	4	4	4	4	4	4	4	4	...	...	...	1
611	3	3	3	...	...	...	...	...	...	...	...	...	...	0
612	3	3	2	2	3	3	3	2	3	...	...	...	...	1
614	3	3	3	...	...	...	...	...	...	...	...	...	...	1
616	3	3	3	4	3	4	3	3	3	3	4	4	4	1
617	4	3	4	4	4	4	4	4	3	3	3	2	...	1
620	3	4	4	4	3	3	3	4	2	4	2	4	3	2
622	2	2	3	2	3	3	3	4	4	...	...	...	...	2
624	2	2	3	2	...	...	...	...	...	...	...	...	...	1
627	2	2	4	2	...	...	...	...	...	...	...	...	...	2
638	2	3	3	2	4	3	3	...	...	...	...	...	...	1
640	3	2	3	2	2	3	3	2	...	...	...	...	...	2
641	3	3	3	3	3	3	3	3	...	...	...	...	...	1
642	3	3	3	3	2	3	3	4	...	...	...	...	...	2
644	3	3	2	2	2	3	3	...	...	...	...	...	...	0
657	2	2	3	2	3	3	3	4	...	...	...	...	...	1
660	2	3	3	4	4	4	4	5	4	4	3	5	4	2
661	...	2	2	1	...	...	...	...	...	...	...	...	...	1
662	...	...	3	3	3	3	3	...	...	...	...	...	...	1
664	...	2	2	3	3	3	3	2	3	...	...	...	...	1
665	...	3	3	3	3	3	3	4	3	3	...	...	...	2
666	...	3	3	3	3	3	3	4	...	...	...	...	...	2
668	...	3	3	2	2	3	3	4	...	...	...	...	...	1
669	...	3	3	2	2	3	3	4	...	...	...	...	...	1
671	...	2	3	2	3	3	1	3	3	3	...	...	...	2
672	...	3	3	2	2	3	...	...	...	...	...	...	...	1
673	...	3	3	1	2	3	...	...	...	...	...	...	...	2



TABLE 1 (Continued)

Cow no.	Inspector and date													
	R 5-42	R 10-42	S 1-43	R 5-43	T 7-43	U 10-43	U 1-44	V 4-44	W 12-44	X 4-45	Y 12-45	T 5-46	Z 12-46	Range
674	.....	4	3	3	3	3	3	4	.....	.....	.....	.....	.....	1
675	.....	3	3	3	2	3	2	2	.....	.....	.....	.....	.....	1
676	.....	4	3	3	2	2	2	3	.....	.....	.....	.....	.....	2
677	.....	2	2	2	2	2	3	.....	.....	.....	.....	.....	.....	0
678	.....	2	2	2	2	2	3	.....	.....	.....	.....	.....	.....	2
679	.....	3	2	1	1	3	2	2	.....	.....	.....	.....	.....	1
680	.....	3	2	3	2	3	2	2	.....	.....	.....	.....	.....	2
681	.....	2	3	2	2	4	3	2	.....	.....	.....	.....	.....	3
683	.....	.....	3	3	3	.....	.....	.....	.....	.....	.....	.....	.....	3
684	.....	.....	3	3	3	.....	.....	.....	.....	.....	.....	.....	.....	3
685	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
686	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
687	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
688	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
689	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
690	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
691	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
692	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
693	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
695	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
698	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
699	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
700	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
701	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
702	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
703	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
704	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
705	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
707	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
714	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
715	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
716	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
719	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
732	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
737	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
742	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
744	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
747	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
754	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3
755	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3

\* Rating code: 5, Excellent; 4, Very Good; 3, Good Plus; 2, Good; 1, Fair.

each instance the type rating was given by an official inspector appointed by the Ayrshire Breeders' Association, who also indicated on a scorecard the main criticisms of each animal classified. The inspectors had no knowledge of any previous ratings on the animals until they had rated each animal and completed their work. The type rating standards of the official type classification program of the Ayrshire Breeders' Association—Excellent, Very Good, Good Plus, Good, and Fair—were used as the standard grades. In order to facilitate the analysis of the data, the official grades were coded, using 5 for Excellent, 4 for Very Good, 3 for Good Plus, 2 for Good, and 1 for Fair.

#### RESULTS

*Variations in individual ratings.* Eighty animals were officially rated three times or more. These ratings are shown in table 1, along with the dates classified, the inspector, and the range in the classification ratings for each cow.

A summary for the table shows that seven animals or 8.8 per cent were rated the same each time they were classified, 47 or 58.7 per cent had a range of one grade, 23 or 28.8 per cent had a range of two grades, and 3 or 3.7 per cent varied three grades. These results indicate that approximately one-third of the 80 head varied two or more grades when classified an average of five times over a period of 4.5 years by different inspectors. A further examination of the data was made to determine the major reasons for variations in type ratings.

*Correlation between type ratings of the same cow when classified by the same inspector and by different inspectors.* The relationships (repeatabilities) between ratings given a cow by the same inspector and between ratings given a cow by different inspectors were computed after the data first were analyzed by the analysis of variance (3). The mean squares for between cows and within cows were obtained and the components of variance for each mean square were determined. This analysis is shown in table 2, where the variation in type ratings of 53 cows classified by inspector R has been analyzed into differences between cows and within cows and the two components of variance, *A* and *B*, are calculated. The component of variance *A* is the variance caused by differences between ratings on the same cow, while *B* represents the additional variation that can be ascribed to differences between cows. The ratio  $\frac{B}{A+B}$  represents the average correlation between the ratings on each cow classified by R and is 0.73. Inspector R classified the cows in May, 1942, October, 1942, and May, 1943.

Similarly, the correlations of repeatabilities of ratings of the same cow when classified by the same inspector were 0.82 and 0.62 for inspectors U

TABLE 2

*Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by inspector E*

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows ...	52	107.2	2.062	$A + 2.47 B$	$B = 0.727$
Within cows .....	78	20.8	0.267	$A$	$A = 0.267$
Total .....	130	128.0	0.985	$A + 0.988 B$	$A + B = 0.994$
				$\frac{B}{A + B} = 0.731$	

\*Coefficients for the components of variance were computed by the method used by Dickerson (1).

and T, respectively, based on 42 and 9 cows. The classifications by inspector U were made in October, 1943, and January, 1944, while inspector T rated the cows in July, 1943, and May, 1946.

In table 3 the analysis of variance was used on the ratings of cows classified two or more times by different inspectors. In this analysis only the first rating placed on an animal by an inspector was used, and any later ratings given to the cow by the same inspector were omitted. The repeatability of ratings for these 101 cows was 0.55.

Tables 1, 2, and 3 indicate that there is a considerable amount of variation in the type ratings of individual Ayrshire cows. Some of the possible reasons for these variations, other than the difference in inspectors, were studied.

*Effect of age on variation of type ratings.* From observation it appeared that as cows advanced in age there was a tendency for the inspectors to raise their rating. In other words, it seemed that when an animal was classified at an early age, the inspector would rate the animal a grade lower if there was any doubt in his mind, because the animal always could be raised at a subsequent reclassification, but never officially lowered. Table 4 gives the average rating of all the cows classified at the ages of 2 to 11 years, inclusive.

TABLE 3

*Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by different inspectors*

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows ...	100	255.7	2.557	$A + 4.446 B$	$B = 0.484$
Within cows .....	348	140.1	0.403	$A$	$A = 0.403$
Total .....	448	395.8	0.883	$A + 0.992 B$	$A + B = 0.887$
				$\frac{B}{A + B} = 0.546$	

\*Coefficients for the components of variance were computed by the method used by Dickerson (1).



TABLE 4  
Average type rating by age for all animals classified between the  
ages of 3 to 11 years, inclusive

Age classified	No. of animals	Av. rating
2	37	2.27
3	204	2.83
4	127	3.01
5	82	3.35
6	48	3.48
7	33	3.61
8	32	3.69
9	22	3.91
10	23	4.26
11	18	4.06

This table might lead one to believe that as the animals advanced in age the rating went up, but the factor of selection must be considered. The better type animals, as a rule, were kept in the herd longer, and those cows that were classified at 5 years and over were a select group of individuals. Averages as given in this table do not present an accurate picture of the effect of age on the variation of individual type ratings.

A more accurate picture is presented when the effect of age on type rating is studied within the same animals. In this study 65 females were included that were classified numerous times as 3-, 4-, and 5-year-olds. Thirty of the same females were classified several times as 4- and 5-year-olds, and 27 of them classified several times as 3-, 4-, and 5-year-olds. The results of this study are presented in table 5 and indicate that the change in type rating due to advancement in age is not great but is statistically significant between the ages of 4 and 5. More years of results will be needed to make the same determinations for older animals.

*Effect of stage of lactation on variation of type ratings.* During each calving interval a cow usually undergoes a constant change as far as her physical appearance is concerned. In the majority of cases dairy cows put on flesh during their dry period and when they come into production they are usually well covered with flesh and have a certain bloom that they lose during a heavy-producing lactation. The additional fleshing may serve to

TABLE 5  
Average type rating by age within cows

Age classified	No. of animals	Av. rating
3	65	2.94
4	65	2.90
4	30	3.05
5	30	3.24
3	27	2.98
4	27	3.02
5	27	3.21

cover up certain faults; on the other hand, it may cause certain cows to appear to be lacking in dairy temperament. The appearance of the udder also changes constantly.

In an effort to determine some specific measure of the effect of stage of lactation on the variation of type ratings of the same cow, an analysis was made of the ratings of 63 animals classified 137 times between freshening and the fourth month of lactation, inclusive, 140 times between the fifth and the ninth month of lactation, inclusive, and 173 times between the tenth month of lactation and the next freshening period. The average of the ratings made between freshening and the fourth month, inclusive, was 3.27; between the fifth and the ninth month, 3.08; and from the tenth month of lactation to the next freshening period, 3.35. These differences are small, but are statistically significant when either the early or the last part of the lactation is compared with the middle segment. The data suggest that a little higher rating is obtained when animals are classified shortly before or after freshening, rather than in the middle part of their lactation.

As yet data are insufficient to make monthly comparisons of ratings throughout the lactation of a number of animals, which would be most desirable. Individual differences are great when cows are compared as to their changes in type conformation throughout a lactation period, and averages such as the above may cover up some of those wide individual differences. A photographic record kept on many of the cows that were classified at various times throughout their lactation is valuable and brings out very clearly how greatly some cows change in appearance, not only with stage of lactation but also with age.

#### DISCUSSION

The results of the project thus far seem to indicate quite definitely that the variations in type ratings of individual Ayrshire cows are considerable over a period of years. These variations are due to several causes, among which are age, stage of lactation, degree of fleshing of the animal, and the differences in inspectors. However, from an analysis of the data, along with the criticisms made of each cow by each inspector at each classification, most of the wide variations in type ratings of individual cows apparently could be attributed to inability of inspectors to attach equal significance to certain faults of a cow. This seemed particularly true when the animals were criticized for defects in udder, feet, and legs. For example, 22 of the 26 cows that had a range of two or more grades changed at least two official grades in two consecutive classifications (an interval of 3 to 6 months). For 19 of these animals the main faults recorded were crooked legs, bad feet, or udders defective in either shape or attachment. As a general rule, the inspectors agreed very closely as to their appraisal of faults other than those found in the feet, legs, and udders.

These results indicate that uniform cuts for the more important defects in feet, legs, and udder should be adopted. Perhaps this can best be accomplished through a series of pictures of cows that exemplify pictorially the type and seriousness of the defects involved. Then perhaps definite point cuts could be determined for the various types of faulty legs, feet, and udders, and these cuts applied by each inspector.

Obviously, the standardizing of the human factor is still the most perplexing problem in type classification work, and consequently, by deliberately planning to use the largest possible number of inspectors, variations in the results were invited. Type rating technique has been and is being improved and, no doubt, will continue to improve. However, the results of this experiment are quite similar to those found by Johnson and Lush (2) in their study of type in Holstein-Friesians,—namely, that two or more type ratings of an individual cow are a far more accurate guide to her true type conformation than is one official rating which can never be lowered irrespective of future conformation changes.

#### SUMMARY

Eighty Ayrshire cows at the West Virginia Agricultural Experiment Station were rated for type an average of five times over a period of 4.5 years by nine different inspectors. A summary of these results shows that 54 animals or 67.5 per cent had a range of one grade or less, while 26 or 32.5 per cent had a range of two or three grades.

The relationships (repeatabilities) between ratings given a cow by the same inspector at different times were calculated for three different inspectors and found to be 0.73, 0.82, and 0.62, while the repeatability of ratings given the same cows by different inspectors was calculated to be 0.55.

Type ratings were found to be somewhat higher on older cows and, in addition, were higher on cows classified during the first 3 months of a lactation or the last 2 months as compared with the middle segment of the lactation.

It is concluded that several type ratings on an individual cow form a far more accurate guide to her true type conformation than does one official rating which can never be lowered.

#### ACKNOWLEDGMENTS

The authors are indebted to Mr. C. T. Conklin, Secretary of the Ayrshire Breeders' Association, for his assistance in this project and for making available official inspectors for each herd classification.

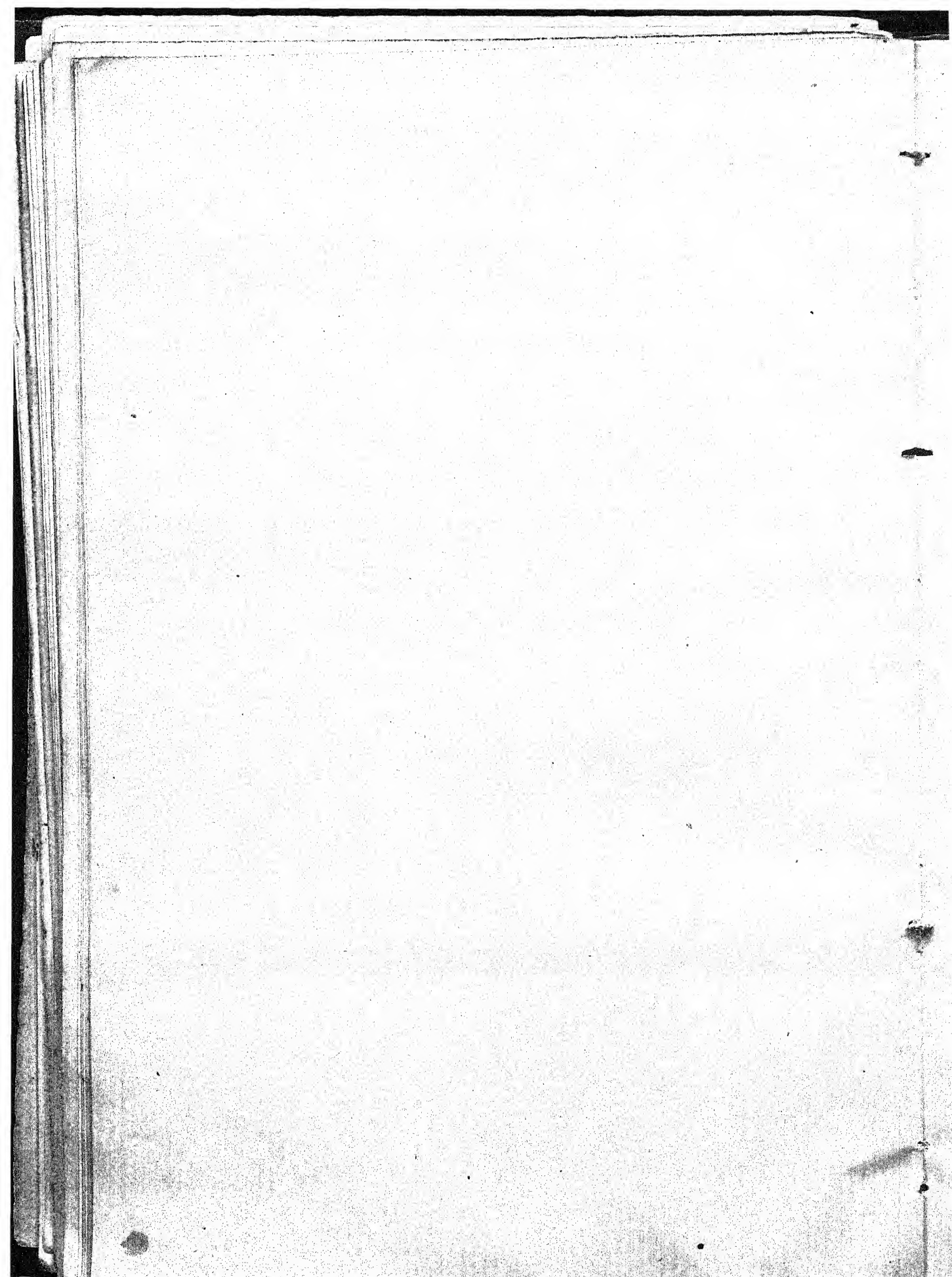
They also express their appreciation to G. A. Bowling, Secretary of the Purebred Dairy Cattle Association, who initiated the Project and collected a considerable portion of the data. They also wish to thank Dr. H. O. Henderson, Head, Department of Dairy Husbandry, West Virginia University,



for his assistance in setting up and carrying out the project and for his valuable suggestions during the preparation of this manuscript.

## REFERENCES

- (1) DICKERSON, G. E. Experimental Design for Testing Inbred Lines of Swine. *J. Animal Sci.*, 1: 326-341. 1942.
- (2) JOHNSON, L. E., AND LUSH, J. L. Repeatability of Type Ratings in Dairy Cattle. *J. Dairy Sci.*, 25: 45-56. 1942.
- (3) SNEDECOR, G. W. Statistical Methods. 4th Ed. Iowa State College Press, Ames, Iowa. 1946.



## THE UTILIZATION OF LACTOSE BY THE DAIRY CALF FED NORMAL OR MODIFIED MILK DIETS<sup>1</sup>

JORGE ROJAS, B. S. SCHWEIGERT, AND I. W. RUPEL

*Department of Dairy Husbandry and Department of Biochemistry and Nutrition,  
Agricultural and Mechanical College of Texas, College Station*

Extensive studies with adult rats have shown that the addition of fat to skim milk diets markedly reduces the galactosuria observed when skim milk plus iron, copper, and manganese is fed as the sole diet (3, 8, 9). Little is known of the effect of fat on the utilization of lactose by the young calf or by other species. Results obtained on one calf and one pig (9) indicate that these animals perform in a manner similar to the rat. Both of these animals were found to utilize lactose completely when a whole milk diet was fed; however, after 2 weeks of skim milk feeding, considerable amounts of galactose were found in the urine. The galactose excretion apparently could be reduced to normal levels when 4 per cent corn oil was fed in addition to skim milk.

It was of importance, therefore, to extend these observations on the utilization of milk sugar by the young calf. The absorption and assimilation of lactose was studied when skim milk, whole milk, or modified whole milk diets were fed.

### EXPERIMENTAL PROCEDURE

Ten male calves were used in these experiments, six Holsteins and four Jerseys. As these animals became available, they were fed either whole milk or skim milk diets. The group fed whole milk was composed of four calves, three Holsteins and one Jersey. Two of these animals received whole milk for the entire experimental period of 42 days, one calf was changed to a skim milk diet after 18 days, and the remaining calf was removed from the experiment after receiving whole milk for 12 days.

After the calves were taken from their dams, a daily milk allowance of 6 lb. for Jerseys and 8 lb. for Holsteins was pail-fed. The allowances were increased during the experiment according to the performance of the individual calf.

The group fed skim milk was composed of six calves, three Holsteins and three Jerseys. Two of these calves received a specially prepared low-fat colostrum for the first 4 days and skim milk thereafter; two suckled their dams for the first 24 hours; two received no colostrum and were fed skim milk from birth. Skim milk allowances were the same as for whole milk for the first 2 or 3 days, but were increased thereafter to compensate partially for the lower energy value of the skim milk.

Received for publication October 3, 1947.

<sup>1</sup> Some of the data were taken from a thesis submitted by Jorge Rojas in partial fulfillment of the requirements for a Master of Science degree.



The skim milk diet was supplemented with 25,000 USP units of vitamin A per day by capsule as fish-liver oil and with approximately 1,000 units of vitamin D (viosterol). Since several investigators have shown that milk is low in iron, copper, manganese, and magnesium (1, 5, 6), the rations were supplemented with 7, 0.7, 0.6, and 43 mg., respectively, of these elements per pound of milk. Cases of scours or other digestive difficulties were treated successfully with 0.25 ounce of sulfaguanidine *per os* daily for 4 days.

All calves were kept in individual stalls with straw bedding, except during the days when urine collections were made. The urine was collected

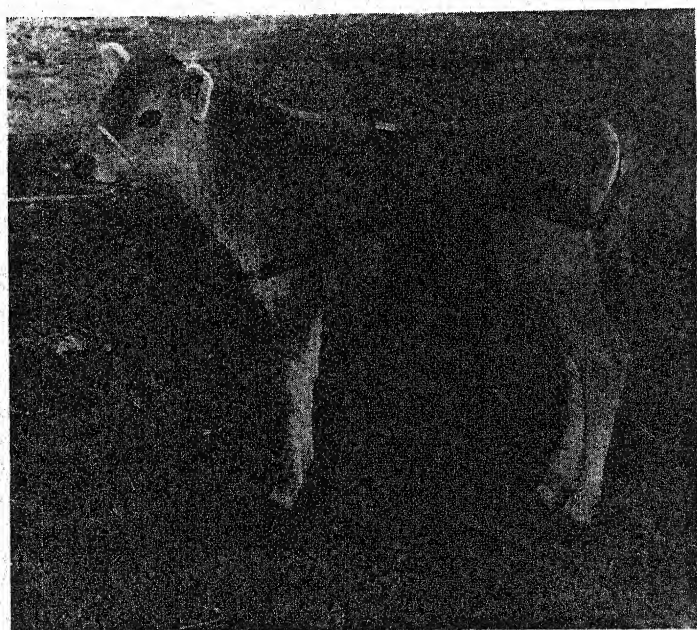


FIG. 1. Apparatus used for making quantitative urine collections from young dairy calves. The apparatus is fastened firmly in order to avoid losses of urine when voided and can be adjusted according to the size of the animal. The animal is placed in a close-fitting elevated crate when urine collections are made, and the hose of the apparatus is passed through a hole in the bottom of the crate and fastened to a bottle placed under the crate.

for 24 hours at regular intervals. An apparatus and crate were designed to obtain quantitative urine collections. The apparatus used is shown in figure 1. The volume of urine voided was recorded and aliquots taken for sugar analyses.

Sugar determinations were made by the Shaffer-Hartmann method (10). The values for sugar were calculated by the use of the Munson-Walker tables (7) with the factor 1.22 to convert glucose values to galactose. It is recognized that urine contains reducing substances other than sugars which

TABLE 1  
*Effect of feeding whole milk on the urinary excretion of galactose*

Periods	Days	Whole milk fed (lb. per day)	No. of calves	No. of collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	$\frac{\text{Galactose excreted}}{\text{Lactose ingested}} \times 100$
I	1-6	7.3	5	6	363.0	5,580	3.4*
II	7-12	7.8	4	8	131.4	3,142	1.8
III	13-18	9.0	3	4	118.0	3,112	1.5
IV	19-24	8.2	2	4	107.8	2,448	1.3
V	25-30	8.5	2	4	118.1	3,071	1.6
VI	31-36	9.0	2	4	81.5	2,218	1.1
VII	37-42	8.1	2	4	140.1	3,433	1.9

\*Holstein calf no. 1139 excreted 12,274 g. galactose during the first collection.

may influence the values obtained. The results are expressed as galactose in all cases, although its presence was not confirmed by osazone tests. The actual values, therefore, cannot be interpreted unequivocally as galactose, but they serve as a useful measurement for comparing the effects of different dietary regimens, as has been widely used by other investigators. The accuracy of the method as applied to urine was checked, and recovery values for galactose added to urine were satisfactory, all within the range of  $\pm 5$  per cent of the theoretical.

#### RESULTS AND DISCUSSION

Previous reports on the utilization of lactose by rats fed skim milk diets were checked. Adult rats fed a skim milk diet for 2 weeks excreted an

TABLE 2  
*Effect of feeding skim milk on the urinary excretion of galactose*

Periods	Days	Skim milk fed (lb. per day)	No. of calves	No. of urine collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	Galactose excreted Lactose ingested $\times 100$
I	1-6	8.2	5	9	182.8	3.030	1.6
II	7-12	9.5	6	12	134.9	2.609	1.3
III	13-18	10.4	6	12	106.6	2.906	1.2
IV	19-24	11.6	7	14	121.2	4.149	1.6
V	25-30	11.7	6	10	120.8	3.319	1.3
VI	31-36	12.1	6	10	132.0	4.680	1.7
VII	37-42	12.3	5	8	115.4	4.751	1.7

average of 20 per cent of the ingested galactose. Substituting whole milk for skim milk in the diet reduced the galactose excretion to 2 per cent of the intake. These results confirm those of other investigators (3, 8, 9).

Summaries of the results for the groups of calves fed whole milk and skim milk diets are shown in tables 1 and 2. These data are subdivided into 6-day periods, which show that the amounts excreted did not increase as the experiments progressed. These results do not agree with those of Schantz *et al.* (9). In no case were values found for galactose excretion that approached the figure of 16 per cent of the intake reported by these investigators. The data show that only a small percentage of the galactose ingested was excreted in the urine, regardless of the dietary treatment used. Furthermore, no difference was observed in the values for galactose excretion between calves maintained on whole milk or skim milk diets for periods up to 42 days.



An inspection of the data for each individual animal failed to reveal any appreciable differences from the data shown as averages for all animals within each group. In some instances, an animal would show a sudden rise in urinary galactose (from 1 to 4 per cent of the intake) but immediately the amount excreted dropped to what can be considered normal. The variation that occurred in urinary galactose from day to day for individual

TABLE 3  
*Effects of feeding additional lactose to calves receiving skim milk diets*

Days on experiment	Ration per calf per day	Total galactose excreted (g. per day)	Galactose excreted $\times 100$ Lactose ingested	Health remarks
(Holstein calf no. 895)				
38-42	12 lb. skim milk	2.938*	1.1	Healthy appearance
44	12 lb. skim milk 320 g. lactose	24.832	4.2	Diarrhea
46	12 lb. skim milk 320 g. lactose	34.366	5.8	Diarrhea
48	12 lb. skim milk	9.706	3.6	Improved
51	12 lb. skim milk	9.034	3.3	Improved
(Jersey calf no. A42)				
32-39	10 lb. skim milk	6.653†	2.9	Healthy appearance
40	10 lb. skim milk 250 g. lactose	19.458	4.1	Diarrhea
42	10 lb. skim milk 250 g. lactose	36.017	7.6	Diarrhea
44	10 lb. skim milk	18.693	8.2	Improved
46	10 lb. skim milk	5.849	2.6	Improved
48	10 lb. skim milk	11.792	5.2	Improved

\* Average for 2 collections.

† Average for 3 collections.

animals and between different animals can be explained at least in part by variations in the volume of urine voided. This variation was appreciably less when the values were calculated for 100 ml. of urine. The percentage excreted was not dependent upon the amount of milk consumed. The calf maintained on whole milk for 18 days and subsequently on skim milk for 24 days failed to show any difference in the per cent of lactose excreted as galactose for the two periods (an average of 1.9 per cent when whole milk was fed and 1.7 per cent for the period when skim milk was fed).

Another calf 2 months of age that had been receiving hay and grain and subsequently was fed only skim milk for 4 weeks did not show any increase in sugar excretion when the latter feeding regimen was used.

Several fecal samples were collected to determine whether the lactose was being absorbed effectively, particularly by calves that were scouring. No appreciable quantities of reducing substances were detected in these samples; therefore, it appears that the calves were assimilating the ingested sugar efficiently.

In later phases of the work, after two of the calves had been fed skim milk for 42 days, the lactose content of their ration was doubled by the addition of lactose to the skim milk in order to determine the effect of increasing the percentage of lactose ingested on its utilization by the calf. The results obtained with these calves are shown in table 3. Addition of lactose to the milk decreased the percentage of sugar utilized by the animal. One of the calves excreted approximately 16 per cent of the ingested galactose. This figure approaches that reported by Schantz *et al.* (9) with skim milk feeding. Within a few hours after the lactose-enriched milk was fed, both calves were afflicted with diarrhea. This symptom has been observed in rats receiving high-lactose diets (2, 4). Additional work on the effect of feeding equivalent amounts of other carbohydrates would be valuable in order to determine whether the rise in sugar excretion occurs only with the ingestion of lactose.

Other observations on the performance of the calves fed whole milk or skim milk diets were of interest. Calves fed the skim milk diets made slower gains, undoubtedly due largely to the reduced caloric intake. Cases of scours occurred in calves fed skim milk as well as those fed whole milk; however, with the latter, a milder form was encountered. Most cases of scours responded satisfactorily to treatment with sulfaguanidine.

The data obtained in these experiments show that the young calf up to 3 months of age does not excrete appreciable quantities of galactose in the urine when receiving either whole milk or skim milk diets. Apparently the absence of fat in the diet does not interfere with the utilization of milk sugar by the young calf.

The work with the rat has been conducted with older animals, and no observations are available on the ability of suckling rats to utilize lactose when fed skim milk diets. Present results with calves suggest that the very young animal, being adapted to the milk diet, utilizes lactose efficiently. Further studies are needed to determine the difference, if any, between the very young and the older bovine in the utilization of lactose.

#### SUMMARY

1. The effect of feeding whole milk or modified milk diets to dairy calves on the amount of sugar excreted in the urine was determined. Equipment was designed for quantitative urine collection.

2. Calves fed whole milk or skim milk for periods up to 42 days utilized the dietary lactose very effectively. No difference was observed in the galactose excretions for the two groups. The amount excreted, measured as galactose, ranged from 1.1 to 3.4 per cent of the total lactose ingested.

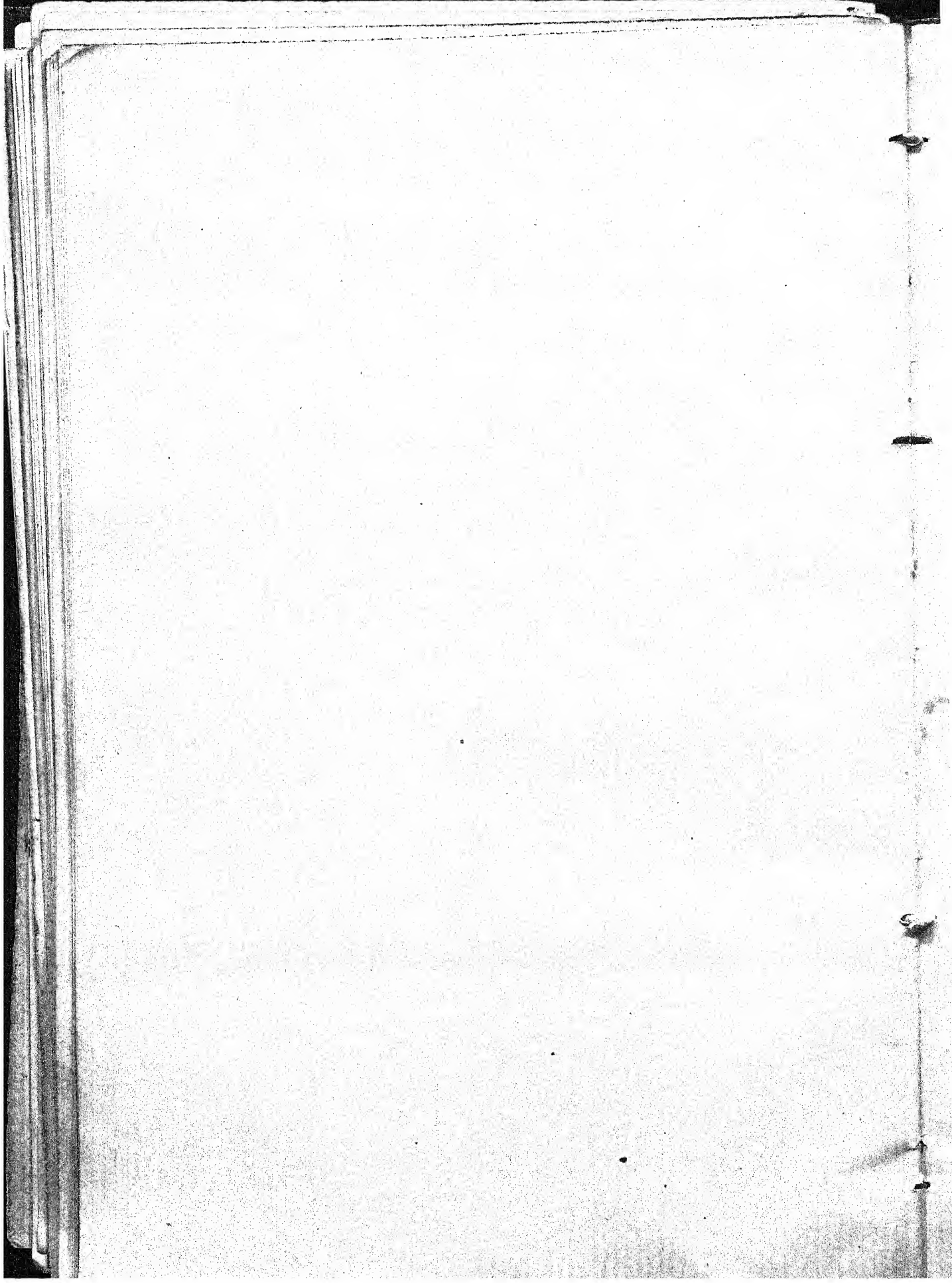
3. When the lactose content of the milk was doubled, the result was an increase in urinary galactose equivalent to 8 per cent of the lactose ingested. The galactosuria was accompanied by diarrhea and unthriftiness of the animals.

The authors are indebted to Dr. F. W. Hill, Western Condensing Company, Appleton, Wisconsin, for supplying some of the lactose used in this study; to Mead, Johnson and Co. for supplying the vitamin D concentrate; and F. E. Booth Company for supplying some of the vitamin A concentrate used in this study. Mr. F. W. Taylor assisted with the care of the animals as part of a collaborative research program on the utilization of vitamin A and carotene by calves fed whole milk or modified milk diets.

#### REFERENCES

- (1) DUNCAN, C. W., HUFFMAN, C. F., AND ROBINSON, C. S. Magnesium Studies in Calves. I. Tetany Produced by Ration of Milk or Milk with Various Supplements. *J. Biol. Chem.*, 108: 35-44. 1935.
- (2) ERSHOFF, B. H., AND DEUEL, H. J., JR. Inadequacy of Lactose and B-Lactose as Dietary Carbohydrates for the Rat. *J. Nutr.*, 28: 225-234. 1944.
- (3) GEYER, R. P., BOUTWELL, R. K., ELVEHJEM, C. A., AND HART, E. B. The Effect of Fat on the Utilization of Galactose by the Albino Rat. *J. Biol. Chem.*, 162: 251-259. 1946.
- (4) HANDLER, P. The Biochemical Defect Underlying the Nutritional Failure of Young Rats on Diets Containing Excessive Quantities of Lactose or Galactose. *J. Nutr.*, 33: 221-233. 1947.
- (5) HUFFMAN, C. F., CONLEY, C. L., LIGHTFOOT, C. C., AND DUNCAN, C. W. Magnesium Studies in Calves. II. The Effect of Magnesium Salts and Various Natural Feeds upon the Magnesium Content of Blood Plasma. *J. Nutr.*, 22: 609-620. 1941.
- (6) KEMMERER, A. R., ELVEHJEM, C. A., HART, E. B., AND FARGO, J. M. The Nutritive Value and Efficiency of Mineralized Milk. *Am. J. Physiol.*, 102: 319-324. 1932.
- (7) MATHEWS, W. P. *Physiological Chemistry*. 5th Ed. William Wood and Co., New York. 1930.
- (8) NIEFT, M. L., AND DEUEL, H. J., JR. The Effect of Fat on the Absorption and Utilization of Galactose by the Rat. *J. Biol. Chem.*, 167: 521-525. 1947.
- (9) SCHANTZ, E. J., ELVEHJEM, C. A., AND HART, E. B. The Relation of Fat to the Utilization of Lactose in Milk. *J. Biol. Chem.*, 122: 381-390. 1938.
- (10) SHAFFER, P. A., AND HARTMANN, A. F. The Iodometric Determination of Copper and Its Use in Sugar Analysis. II. Methods for the Determination of Reducing Sugars in Blood, Urine, Milk, and Other Solutions. *J. Biol. Chem.*, 45: 365-390. 1921.





## ASSOCIATION ANNOUNCEMENT

ANNUAL MEETING—June 14, 15, and 16, 1948

at

UNIVERSITY OF GEORGIA

Athens, Georgia

### FIRST CALL FOR PAPERS

This announcement is to remind members who plan to present original papers at the Annual Meeting that titles should be sent to the member of the Program Committee who represents the section before which the paper is to be given. It is important that all titles must reach the committee before March 1, 1948. Earlier receipt will assist greatly in arranging the best possible program and help to avoid the last mad rush when it is so easy to make mistakes.

Address all communications regarding general plans for the Annual Meeting to the Chairman of the General Program Committee.

#### Manufacturing Section:

P. R. Elliker  
Bacteriology Department  
Oregon State College  
Corvallis, Oregon

#### Production Section:

George H. Wise  
Dairy Husbandry Department  
Iowa State College  
Ames, Iowa

#### Extension Section:

E. H. Loveland  
Extension Dairyman  
University of Vermont  
Burlington, Vermont

#### General Program Chairman:

Fordyce Ely  
Dairy Husbandry Section  
University of Kentucky  
Lexington 29, Kentucky

OFFICERS AND COMMITTEES OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION

## ASSOCIATION OFFICERS

PAUL H. TRACY, Illinois, *President*W. E. PETERSEN, Minnesota, *Vice President*      F. E. NELSON, Iowa, *Journal Editor*R. B. STOLTZ, Ohio, *Secretary-Treasurer*

## ASSOCIATION DIRECTORS

C. Y. CANNON, Iowa  
K. L. TURK, New York  
S. J. BROWNELL, New York  
P. F. SHARP, CaliforniaW. V. PRICE, Wisconsin  
J. H. HILTON, North Carolina  
FORDYCE ELY, Kentucky

## ASSOCIATION COMMITTEES

## BORDEN AWARD COMMITTEES

*Production*C. W. TURNER, Missouri, *Chairman*

G. M. CAIRNS, Maryland      GLENN SALISBURY, Illinois

*Manufacturing*B. E. HORRALL, Indiana, *Chairman*

B. E. GOODALE, South Carolina      I. A. GOULD, Maryland

## RESOLUTIONS COMMITTEE

W. H. E. REID, Missouri, *Chairman*MASON CAMPBELL, Rhode Island      J. H. HILTON, North Carolina  
E. L. FOUTS, Florida      GEORGE PUTNAM, Illinois

## NOMINATING COMMITTEE

G. H. WILSTER, Oregon, *Chairman*F. J. DOAN, Pennsylvania      J. W. LINN, Kansas  
FORDYCE ELY, Kentucky      G. C. NORTH, Illinois

## PROGRAM COMMITTEE

FORDYCE ELY, Kentucky, *Chairman*P. R. ELLIKER, Oregon      G. H. WISE, Iowa  
E. H. LOVELAND, Vermont      JENNINGS B. FRYE, Jr., Georgia



## JOURNAL MANAGEMENT COMMITTEE

W. E. PETERSEN, Minnesota, *Chairman*

P. R. ELLIKER, Oregon

F. E. NELSON, *ex-officio*, Iowa

T. S. SUTTON, Ohio

R. B. STOLTZ, *ex-officio*, Ohio

## AUDITING COMMITTEE

R. A. LARSON, Ohio, *Chairman*

D. V. JOSEPHSON, Ohio

W. E. KRAUSS, Ohio

## NECROLOGY COMMITTEE

G. M. TROUT, Michigan, *Chairman*

H. P. DAVIS, Nebraska

E. O. HERREID, Illinois

## HONORS COMMITTEE

A. C. RAGSDALE, Missouri, *Chairman*

J. A. NELSON, Montana

FORDYCE ELY, Kentucky

## RULES FOR HANDLING AMERICAN FEED MANUFACTURERS AWARD COMMITTEE

C. F. HUFFMAN, Michigan, *Chairman*

J. G. ARCHIBALD, Massachusetts

R. B. BECKER, Florida

## AFFILIATION WITH THE INTERNATIONAL DAIRY FEDERATION COMMITTEE

O. F. HUNZIKER, Illinois, *Chairman*

A. C. DAHLBERG, New York

C. N. SHEPARDSON, Texas

GEORGE HOLM, Washington, D. C.

GEORGE E. RAITHY, Ontario

## OFFICERS AND COMMITTEES OF THE EXTENSION SECTION

## SECTION OFFICERS

E. H. LOVELAND, Vermont, *Chairman*

G. HEEBINK, West Virginia,

C. W. REEVES, Florida,

*Vice Chairman**Secretary*

## SECTION COMMITTEES

*Program Committee*E. H. LOVELAND, Vermont, *Chairman*

G. HEEBINK, West Virginia

F. W. FITCH, Georgia

C. W. REEVES, Florida

*Dairy Records Committee*CHAS. GEARHART, Pennsylvania, *Chairman*

E. J. STALLARD, Wisconsin

ROY JONES, Washington, D. C.

J. D. BURKE, New York

*4-H Club Committee*JOS. NAGEOTTE, Pennsylvania, *Chairman*

GEO. MCINTYRE, Michigan

M. G. REGAN, Missouri

H. A. WILLMAN, New York

RALPH PORTERFIELD, Maryland

*Teaching Methods Committee*I. L. PARKIN, Pennsylvania, *Chairman*

D. L. MURRAY, Michigan

F. W. FITCH, Georgia

HILTON BOYNTON, New Hampshire

## OFFICERS AND COMMITTEES OF THE PRODUCTION SECTION

## SECTION OFFICERS

G. H. WISE, Iowa, *Chairman*

L. A. MOORE, Washington, D. C.,

G. M. CAIRNS, Maryland,

*Vice-Chairman**Secretary*

## SECTION COMMITTEES

*Program Committee*G. H. WISE, Iowa, *Chairman*

L. A. MOORE, Washington, D. C.

D. M. SEATH, Louisiana

*Dairy Cattle Judging Committee*S. M. SALISBURY, Ohio, *Chairman*

ROBERT E. JOHNSON, Connecticut

D. L. FOURT, Idaho

## JOINT COMMITTEES OF THE PRODUCTION AND EXTENSION SECTIONS

*Breeds Relations Committee**Production Section*

W. W. YAPP, Illinois

H. A. HERMAN, Missouri, *Chairman*

D. L. FOURT, Idaho

*Extension Section*

E. C. SCHEIDENHELM, New Jersey

FLOYD ARNOLD, Iowa, *Secretary*

LYNN COPELAND, Tennessee

*Dairy Cattle Breeding Committee**Production Section*

L. O. GILMORE, Minnesota

J. L. LUSH, Iowa

D. M. SEATH, Louisiana

*Extension Section*

S. J. BROWNELL, New York

E. J. PERRY, New Jersey, *Chairman*

GEORGE E. PARSONS, Michigan

*Dairy Cattle Health Committee**Production Section*

L. A. MOORE, Washington, D. C.,  
*Chairman*  
 W. E. PETERSEN, Minnesota  
 W. D. POUNDEN, Ohio

*Extension Section*

J. W. LINN, Kansas  
 C. G. BRADT, New York  
 WILLIAM WALKER, Connecticut

## OFFICERS AND COMMITTEES OF THE MANUFACTURING SECTION

## SECTION OFFICERS

PAUL R. ELLIKER, Oregon, *Chairman*

E. M. BARKER, Minnesota, *Vice-Chairman*  
 D. V. JOSEPHSON, Ohio, *Secretary*

## SECTION COMMITTEES

*Standing Committee on Milk*

E. O. HERREID, Illinois, *Chairman*  
 D. V. JOSEPHSON, Ohio  
 BURDET HEINEMANN, Missouri

*Standing Committee on Butter*

G. H. WILSTER, Oregon, *Chairman*  
 D. K. HOWE, Nebraska  
 C. JENSEN, North Dakota

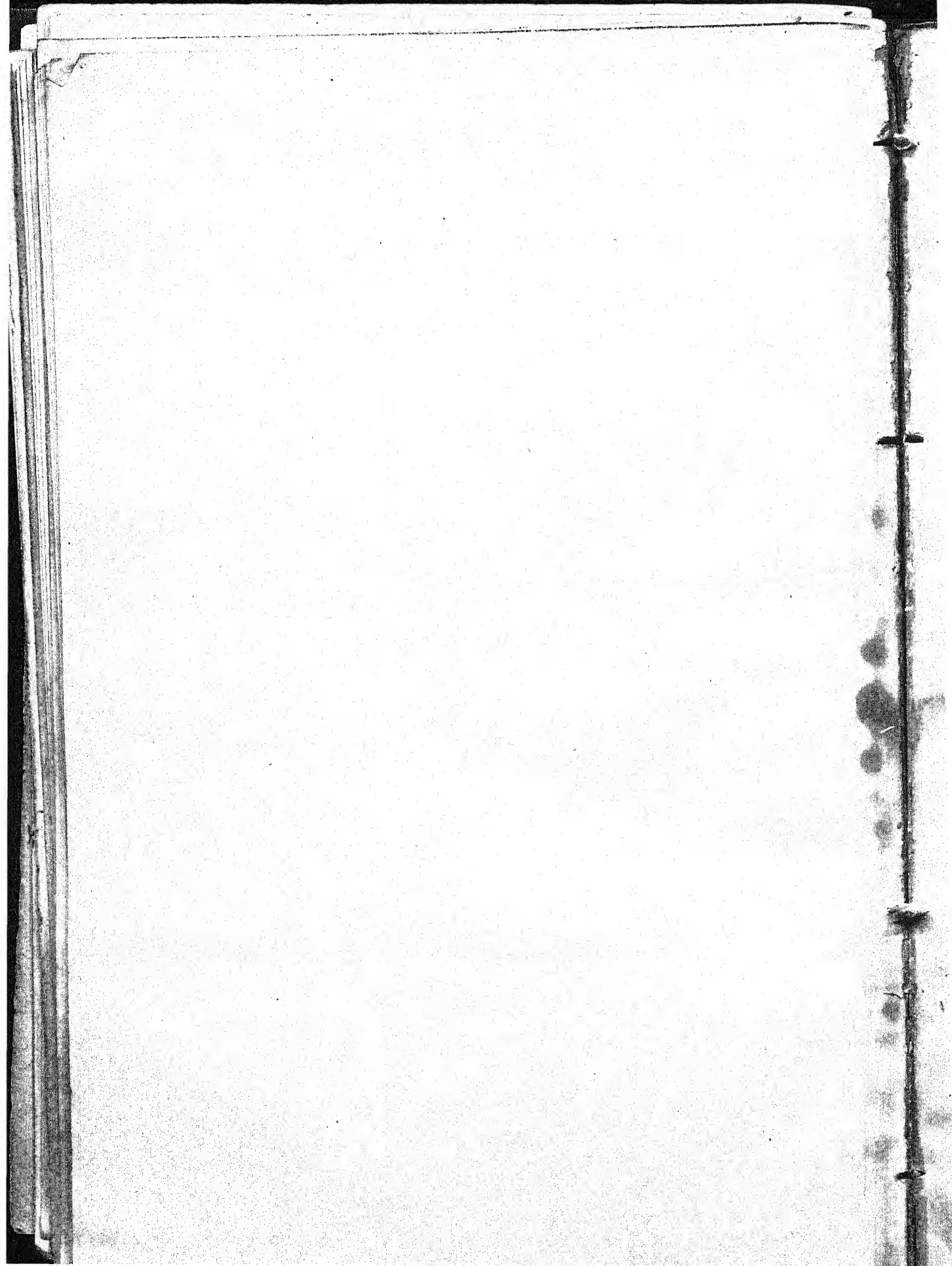
*Standing Committee on Standardization of Tests of Dairy Alkalies and Methods of Reporting Results*

C. W. ENGLAND, Maryland, *Chairman*  
 D. H. JACOBSEN, Chicago  
 C. W. RINK, Pennsylvania

*Standing Committee on Products Judging*

G. M. TROUT, Michigan, *Chairman*  
 P. A. DOWNS, Nebraska  
 C. J. BABCOCK, Washington, D. C.  
 F. H. HERZER, Mississippi  
 E. O. ANDERSON, Connecticut





# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

FEBRUARY, 1948

NUMBER 2

## THE RELATIONSHIP OF MAMMARY DEVELOPMENT AND BODY WEIGHT<sup>1</sup>

J. R. SCHABINGER AND C. B. KNOTT

*Department of Dairy Husbandry, Pennsylvania State College*

A method of selecting dairy calves on the basis of mammary development has been presented by Swett and Matthews (1). The extent of the mammary development of the heifer calf at 3 to 4 months of age has been demonstrated to be in direct relationship with the heifer's later productive ability (1).

This report deals with a study of the relationship of the mammary development to the body weight of Holstein and Guernsey heifer calves at 3 and 6 months of age.

### EXPERIMENTAL PROCEDURE

Nineteen heifer calves (10 Holsteins and 9 Guernseys) were fed six grain rations which resulted in various rates of body growth. The original study involved an investigation into the possibilities of utilizing distillers dried solubles and distillers dried solubles with grains in the rations of dairy calves. While there were no statistically significant differences in the rates of growth of the several groups of calves, considerable variations existed in this respect between calves within the groups. The mammary development of all heifer calves was measured by the technique set forth by Swett and Matthews (1) at 3 and 6 months of age. Body weights were determined at the same ages.

Measurements were made of the width and length of each quarter at 3 months of age. Since the udders were in the half stage when the calves were 6 months of age, only the length of each half and the width of each quarter were measured at this age.

The mean width of the mammary tissue of the four quarters was correlated with body weight at 3 and 6 months. The mean length of the mammary tissue of the four quarters was correlated with body weight at 3 months. However, at 6 months it was necessary to use the mean length of

Received for publication August 13, 1947.

<sup>1</sup> Authorized for publication on August 8, 1947, as Paper no. 1385 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE 1

*Summary of udder measurements of Holstein calves at 3 months of age*

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
285	20.25	194	20.75	94
170	15.25	176	17.25	83
270	17.75	171	22.75	73
186	16.25	165	16.25	80
207	16.50	163	14.75	65
176	14.25	155	15.25	71
187	14.25	153	15.00	68
231	11.75	144	12.75	56
205	15.75	139	15.50	61
212	11.50	123	10.50	46

the mammary tissue of the two halves because the udder development had reached the half-stage. Similar correlations also were made with gain in body weight from 8 days to 3 and to 6 months.

## EXPERIMENTAL RESULTS

The udder measurements and body weights of the ten Holstein calves at 3 months of age are summarized in table 1. These data are presented in order of body weights. The correlation coefficient between the average width of the quarters and body weight was  $+0.844^{**}$ .<sup>2</sup> A correlation coefficient of  $+0.821^{**}$  was determined for the average length of quarters and body weight for these same ten calves. In table 2 are the data for nine Guernsey calves. A correlation coefficient of  $+0.909^{**}$  was found for body weight and the average width of the quarters. For the average length of the quarters and body weight a correlation coefficient of  $+0.917^{**}$  was

TABLE 2

*Summary of udder measurements of Guernsey calves at 3 months of age*

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
200	24.25	191	27.00	99
199	19.5	188	23.5	93
169	12.5	133	12.75	53
227	10.25	131	14.25	52
158	13.00	128	11.25	52
226	8.5	117	9.5	42
153	7.75	109	10.5	46
181	13.75	107	14.5	55
178	6.75	98	..... <sup>a</sup>	34

<sup>a</sup> The glandular tissue was too small to measure accurately.

<sup>2</sup> \* = significant.

\*\* = highly significant.



TABLE 3

*Summary of udder measurements of Holstein calves at 6 months of age*

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
207	46.5	396	77.0	298
270	45.25	378	82.0	280
186	41.25	371	53.0	286
187	31.5	349	67.0	264
231	45.75	340	70.0	252
205	47.5	337	93.0	259
285	41.5	334	69.5	234
170	40.5	333	73.5	240
176	33.75	319	70.5	235
220	51.5	309	75.0	210
212	41.5	273	86.5	196

found. The correlation coefficients were statistically significant for both length and width of the glandular tissue for the Holstein and Guernsey calves.

The summary of udder measurements and body weights for 11 Holstein calves at 6 months of age is presented in table 3. The correlation coefficient for the average width of the glandular tissue and body weight was found to be +0.0836 and -0.29 for the average length of the half stage of the udder and body weight. When tested statistically neither of these correlation coefficients was significant.

The summary of the measurements for eight Guernsey calves at 6 months of age is presented in table 4. The correlation coefficient for the average width of each quarter with body weight was +0.444. However, this coefficient was not significant. The average length of the half stage of the udder and the body weight had a correlation coefficient of +0.834\*. A *t* value of 3.707 was necessary to be highly significant with six degrees of freedom. A value of 3.701 was obtained when the *t* value was determined. Thus the length of the udder and body weight were significantly related.

TABLE 4

*Summary of udder measurements of Guernsey calves at 6 months of age*

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
200	41.5	357	95.0	265
226	53.5	293	89.0	213
227	36.0	286	73.0	207
153	31.75	281	63.5	213
158	31.0	275	60.5	199
169	37.25	274	64.0	194
178	31.25	272	72.0	208
181	32.75	266	58.5	214

Mammary development was correlated with rate of growth as determined by gain in body weight from 8 days to 3 months of age. A coefficient of  $+0.812^{**}$  and  $+0.7707^{**}$  was found for width and length, respectively, for the Holstein calves. Also, with the Guernsey calves, a correlation coefficient of  $+0.9659^{**}$  was calculated for width and  $+0.9756^{**}$  for length of the secretive tissue, and gain in body weight.

No significant relationship was shown to exist between gain in body weight from 8 days to 6 months and udder development at 6 months of age. Correlation coefficients of  $-0.006$  for width and  $-0.2676$  for length of mammary secretive tissue were determined with the Holstein calves, while correlation coefficients of  $+0.345$  (width) and  $+0.2386$  (length) were determined with the Guernsey calves.

#### SUMMARY

Nineteen calves of the Holstein and Guernsey breeds were used to determine the relationship of mammary development to body weight. A highly significant statistical relationship was found to exist between the development of the mammary secretive tissue and body weight of both the Holstein and Guernsey heifer calves at 3 months of age. Highly significant correlations also were found between mammary tissue development and gains in body weight from 8 days to 3 months of age of the heifer calves of both breeds.

The lengths of the secretive tissues of Guernsey calves were related significantly to body weight at 6 months of age; however, there was no correlation in this respect with the Holstein calves at this age. There was no significant correlation between the width of the mammary secretive tissue and body weight of either breed at 6 months of age. No significant correlation was found between the gains in body weight from 8 days to 6 months and mammary development of either breed.

#### REFERENCE

- (1) SWETT, W. W., AND MATTHEWS, C. A. Dairy Cow's Udder Studied to Establish Development Standards. Yearbook of Agriculture. Pp. 175-181. 1934.

## PERMANENCY OF SYNTHETIC ASCORBIC ACID ADDED TO MILK<sup>1</sup>

ARTHUR D. HOLMES AND CARLETON P. JONES  
*Massachusetts Agricultural Experiment Station, Amherst*

Hand (3) and others have shown that the reduced ascorbic acid content of raw, commercial cow's milk decreases rapidly during the first week after it is drawn from the cow. In his study, the average ascorbic acid value for 12 samples of milk was 19 mg. per l. at the beginning of storage at 1° C. and 7 mg. per l. 6 days later. Thus about 63 per cent of the original reduced ascorbic acid had disappeared from the milk during a 6-day storage period. Subsequently, Holmes and Jones (5) determined the loss of reduced ascorbic acid in mare's milk. They found that the rate of disappearance of ascorbic acid from mare's milk was only about one-seventh that reported by Hand for cow's milk. Since the composition of cow's milk and mare's milk is dissimilar in various respects, it is possible that a number of factors may influence the rate of loss of reduced ascorbic acid from the two types of milk. One obvious difference in composition is the amount of reduced ascorbic acid in the original milk. Hand reported that his samples of cow's milk contained from 14.8 to 22.8 mg. of ascorbic acid per l., whereas Holmes and Jones used samples of mare's milk that contained from 86 to 161 mg. of ascorbic acid per l. Accordingly, it was decided to determine the rate of loss of reduced ascorbic acid from cow's milk to which a sufficient amount of synthetic ascorbic acid had been added so that the ascorbic acid content of the milk approximated that of the mare's milk referred to above.

### EXPERIMENTAL PROCEDURE

Since the stability of reduced ascorbic acid had been determined for raw mare's milk, raw cow's milk was used in this study. Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of synthetic ascorbic acid to a liter of milk. After the ascorbic acid was added, the milk was shaken thoroughly. One sample each of milk containing 75 mg. and 150 mg. of added ascorbic acid per l. was prepared per day. The enriched milk was placed in 500-cc. flasks and stored in the dark at 10° C. When the samples were prepared, the flasks were completely filled, but as aliquots were taken day by day for assay, the volume of milk decreased and the volume of atmosphere increased correspondingly. These conditions were the same as for the study of the stability of ascorbic acid in mare's milk and they were similar to the conditions in the average household where milk is stored in

Received for publication September 19, 1947.

<sup>1</sup> Contribution no. 636 of the Massachusetts Agricultural Experiment Station.



the refrigerator and at irregular intervals variable amounts are removed from the milk bottles.

The storage period was 10 days and only one sample of each series was placed in storage at a time. The amount of reduced ascorbic acid in all cases was determined by the method described by Holmes and Jones (5), and bentonite was used for clarification.

#### RESULTS AND DISCUSSION

The average values for the ascorbic acid assays of the two series of samples are reported in figure 1. The samples of the original milk before

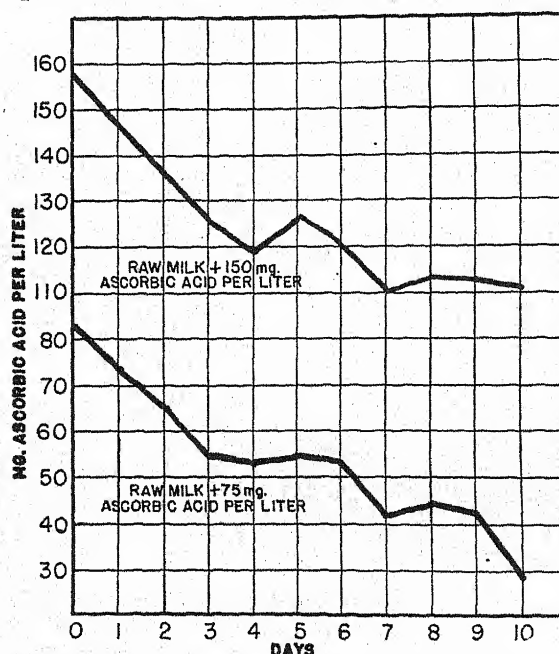


FIG. 1. Rate of loss of synthetic ascorbic acid from cow's milk.

the addition of the synthetic ascorbic acid contained, on an average, 9.5 mg. of reduced ascorbic acid per l. This value is in agreement with 7.5–9.2 mg. per l. reported by Christen and Virasoro (1), 10.8 mg. per l. by Lojander (9), and 12.2 mg. per l. by Mosonyi and Polónyi (10). However, these values for the ascorbic acid content of raw cow's milk definitely are less than those usually reported for milk—i.e., 16.4 mg. per l. reported by Holmes *et al.* (7), 17.1 mg. per l. by Stewart and Sharp (12), 17.4 mg. per l. by Woessner *et al.* (13), 19.7 mg. per l. by Holmes *et al.* (6) and 22.2 mg. per l. by Sharp *et al.* (11).

When the two series of samples of ascorbic acid-enriched milk were placed in storage, their average reduced ascorbic acid contents were 83.0 mg.

and 157.5 mg. per l., respectively. Thus, losses of about 1.7 per cent and 1.2 per cent, respectively, occurred while the milk was being enriched and prepared for study. At this time the milk was exposed to laboratory temperature and full daylight but not to sunshine.

During the first 3 or 4 days of storage, reduced ascorbic acid was lost more rapidly and more consistently than during the remainder of the experimental period. For the series of samples of milk to which 75 mg. per l. of ascorbic acid was added, the loss of ascorbic acid was 34 per cent during the first 3 days or 11 per cent per day, and 33 per cent during the remaining 7 days or 5 per cent per day, with an average loss of 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid was added per l., the loss was 24 per cent for the first 4 days or 6 per cent per day, and 5 per cent for the next 6 days or 1 per cent per day, with an average loss of 3 per cent per day for the 10 days the milk was in storage. These losses are decidedly less than those reported by Gunsalus and Hand (2), who noted a reduction of reduced ascorbic acid of from 14.9 mg. to 1.7 mg. per l. or an average loss of 14.7 per cent per day during 6 days' storage of raw cow's milk. Hand (3) observed a loss of from 19.0 mg. to 7.1 mg. per l. of milk stored 6 days at 1° C., averaging over 10 per cent per day. Kothavalla and Gill (8) reported a loss of 26 per cent of ascorbic acid from cow's milk (Indian) stored at 45° F., or an average of over 8 per cent per day. Thus it appears from the data assembled here that when considerable amounts of synthetic ascorbic acid are added to raw cow's milk, the percentage of loss of ascorbic acid during storage is smaller than for the reduced ascorbic acid naturally occurring in raw cow's milk. It should be noted that, except for the period while the samples were being prepared at room temperature and for short intervals while the aliquots for assay were being withdrawn, the milk was stored in the dark at 10° C. Consequently, in this study as well as in the study of the stability of ascorbic acid in mare's milk, the effect of light and elevated temperatures upon the destruction of the ascorbic acid was kept at a minimum. Holmes and Jones (4) have shown that these factors cause exceedingly rapid destruction of reduced ascorbic acid in cow's milk. Obviously the data assembled here, together with those reported by the cited investigators, are not sufficient to provide a complete understanding of the factors and conditions that influence the rapid destruction of reduced ascorbic acid occurring naturally in cow's milk, or to provide means for preventing the unfortunate loss of this essential vitamin from one of the most valuable human foods.

#### SUMMARY

Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of ascorbic acid to a liter of raw cow's milk. The samples were stored in 500-cc. flasks in the dark at 10° C. As aliquots were removed day by

day for analysis, the volume of milk decreased and the volume of air in the flasks increased correspondingly. For the series of samples of milk to which 75 mg. of ascorbic acid per l. was added, the loss was 11 per cent per day for the first 3 days and 5 per cent per day for the remaining 7 days, or 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid per l. was added, the loss was 6 per cent per day for the first 4 days and 1 per cent per day for the remaining 6 days, or an average of 3 per cent per day for the 10 days of storage.

## REFERENCES

- (1) CHRISTEN, C., AND VIRASORO, E. Determination of Ascorbic Acid by the Methylene Blue Method. *Anales asoc. quim. argentina*, 30: 197-211. 1942. (Chem. Abs., 37: 3839. 1943.)
- (2) GUNSALUS, I. C., AND HAND, D. B. The Use of Bacteria in the Chemical Determination of Total Vitamin C. *J. Biol. Chem.*, 141: 853-858. 1941.
- (3) HAND, D. B. Reduced and Total Vitamin C in Milk. *J. Dairy Sci.*, 26: 7-12. 1943.
- (4) HOLMES, A. D., AND JONES, C. P. Effect of Sunshine upon the Ascorbic Acid and Riboflavin Content of Milk. *J. Nutrition*, 29: 201-209. 1945.
- (5) HOLMES, A. D., AND JONES, C. P. Stability of Reduced Ascorbic Acid in Mare's Milk. *J. Nutrition*, 34: 113-119. 1947.
- (6) HOLMES, A. D., JONES, C. P., WERTZ, ANNE W., AND KUZMESKI, J. W. The Ratio of Ascorbic Acid, Riboflavin, and Thiamin in Raw and Pasteurized Milk. *J. Nutrition*, 26: 337-345. 1943.
- (7) HOLMES, A. D., LINDQUIST, H. G., JONES, C. P., AND WERTZ, ANNE W. Effect of High-Temperature-Short-Time Pasteurization on the Ascorbic Acid, Riboflavin, and Thiamin Content of Milk. *J. Dairy Sci.*, 28: 29-33. 1945.
- (8) KOTHAVALLA, Z. R., AND GILL, H. S. The Effect of Various Factors on the Vitamin C Content of the Milk of Cows and Buffaloes. *Indian J. Vet. Sci.*, 13: 35-43. 1943. (Chem. Abs., 39: 2346. 1945.)
- (9) LOJANDER, W. Variations in the Vitamin C Content of Milk and Factors Contributing to Such Variation. *Acta Soc. Med. Fennicae Duodecim*, 27: 1-10. 1939. (Chem. Abs., 35: 5579. 1941.)
- (10) MOSONYI, J., AND POLONYI, P. Vitamin C Content of Cow's Milk. *Kísérletiügyi Közlemények*, 44: 97-106. 1941. (Chem. Abs., 38: 2757. 1944.)
- (11) SHARP, P. F., GUTHRIE, E. S., AND HAND, D. B. Deaeration as a Means of Retarding Oxidized Flavors and Preserving the Vitamin C of Milk. *Ann. Rept. N. Y. State Assoc. Dairy & Milk Inspectors*, 14: 63-76. 1940.
- (12) STEWART, A. P., JR., AND SHARP, P. F. Vitamin C Content of Market Milk, Evaporated Milk, and Powdered Whole Milk. *J. Nutrition*, 31: 161-173. 1946.
- (13) WOESSNER, W. W., WECKEL, K. G., AND SCHUETTE, H. A. The Effect of Commercial Practices on Ascorbic Acid and Dehydroascorbic Acid (Vitamin C) in Milk. *J. Dairy Sci.*, 23: 1131-1141. 1940.



## SULFAMETHAZINE BLOOD AND MILK CONCENTRATIONS IN DAIRY COWS

PETER H. LANGER, ROBERT L. BURKHART, CHARLES R. SCHROEDER,  
AND MARK WELSH<sup>1</sup>

*Lederle Laboratories Division, American Cyanamid Company,  
Pearl River, New York*

Bovine mastitis continues to be of great economic importance to all concerned with the production of milk. Reliable statistics on the losses attributed to the infection are unavailable, but it is considered to be the most important disease problem of the dairy industry (7). Various sulfonamides have been used in the treatment of mastitis. On the basis of present knowledge concerning the action of the sulfonamides, it would appear that the effectiveness of a sulfonamide depends upon adequate blood levels. The present study was initiated to determine the relationship between blood and milk concentrations of sulfamethazine.

### CAUSES OF MASTITIS

Several species of bacteria may be associated with mastitis, but *Streptococcus agalactiae* has been recovered in the majority of cases. Staphylococci are considered to be second in importance, followed by other species of streptococci, coliform organisms, and corynebacteria (12). The exact manner of transmission of the disease is not known, but the most probable route of infection is through the teat duct. Environmental factors, repeated exposure to highly infective organisms, and injury to the udder, or a combination of factors, all have been held responsible for spread of the disease.

### COMMONLY USED THERAPEUTIC AGENTS

Therapeutic agents have been relied upon to a great degree in the control of mastitis. While immediate infection can be corrected, re-infection cannot be prevented by these measures. However, correct herd management, in conjunction with good treatment procedures when disease does occur, will maintain a productive herd.

*In vitro* and *in vivo* studies have shown that sulfonamides are active against the streptococci, staphylococci, and other species of bacteria occurring in mastitis. In the early days of sulfonamide therapy, sulfanilamide was administered by mouth in the treatment of mastitis, and it continues to be

Received for publication October 2, 1947.

<sup>1</sup> The authors are indebted to Mr. S. C. Griffith, of the Animal Industry Section, for aid in the conduct of the program, and to Mr. S. Senn and Mr. D. W. Gorton, of the Analytical Section, Lederle Laboratories Division, American Cyanamid Company, for sulfonamide analyses.

used by many veterinarians. The reported results (10, 14) are conflicting. However, subsequent experience with sulfanilamide has shown its clinical use to be limited by its rather narrow range of bacteriostatic activity against those organisms other than streptococci which are pathogenic for man and animals.

It has been reported (3, 5, 13) that therapeutic agents for intramammary infusion, with the possible exception of penicillin in sterile water or physiological saline, frequently result in varying degrees of udder irritation, and, in some cases, cause permanent damage, as evidenced by decrease in milk flow and production of abnormal milk. Several investigators attempted to find an agent which would be effective in mastitis when administered parenterally, inasmuch as unsatisfactory results too frequently followed treatment by udder infusion. Because of the success of the therapeutic use of penicillin in human streptococci and staphylococci infections, and the successful use of intramammary infusions of penicillin in mastitis (1, 9, 11), work was undertaken to determine the permeability of the bovine mammary gland to penicillin parenterally administered. Such trials have been disappointing (2, 6, 15, 17), and it was found that penicillin was not present in the milk in detectable amounts or amounts sufficient to affect existing mastitis infection. In this work, however, dosages of penicillin used in cows were not sufficiently great, as judged by amounts needed to control human disease effectively. Watts and McLeod (17) reported the use of doses of 1,000,000 Oxford units, with no diffusion of penicillin in the milk. Obviously, larger dosages, and the frequent administration necessary, generally would be economically unsound.

Welsh *et al.* (18) showed that sulfamethazine, the dimethyl derivative of sulfadiazine, maintained the highest blood concentration of seven sulfonamides tested, over a 24-hour period on a fixed intake. It has been reported to be among the least toxic of the sulfonamides in therapeutic dosages, and its action against both Gram-negative and Gram-positive organisms frequently has been shown. Lately, evidence has been presented that sulfamethazine therapy alone, or in combination with penicillin (8, 16), can correct immediate infection and keep cows in the milking string.

#### EXPERIMENTAL PROCEDURE

Four normal cows were used in these experiments. To permit correct comparisons, the same cows were used in all three tests, with rest periods of 12 and 5 days, respectively, after each trial. Freedom from clinical mastitis at the time of the trial was determined on the basis of udder palpation and physical appearance of the milk by strip cup test.

The cows were maintained under conditions comparable to those of the average farm. Water was continuously available at automatic foun-

tains. Cows regularly were turned out to pasture. They were milked at 12-hour intervals, and, at the time of the trial, they were producing a minimum of 40 lb. of milk per day.

The dosage used throughout the experiment was 1.5 grains per lb. of body weight on the first day, and 1 grain per lb. of body weight on the second day. This dosage was administered in three ways: (a) One daily

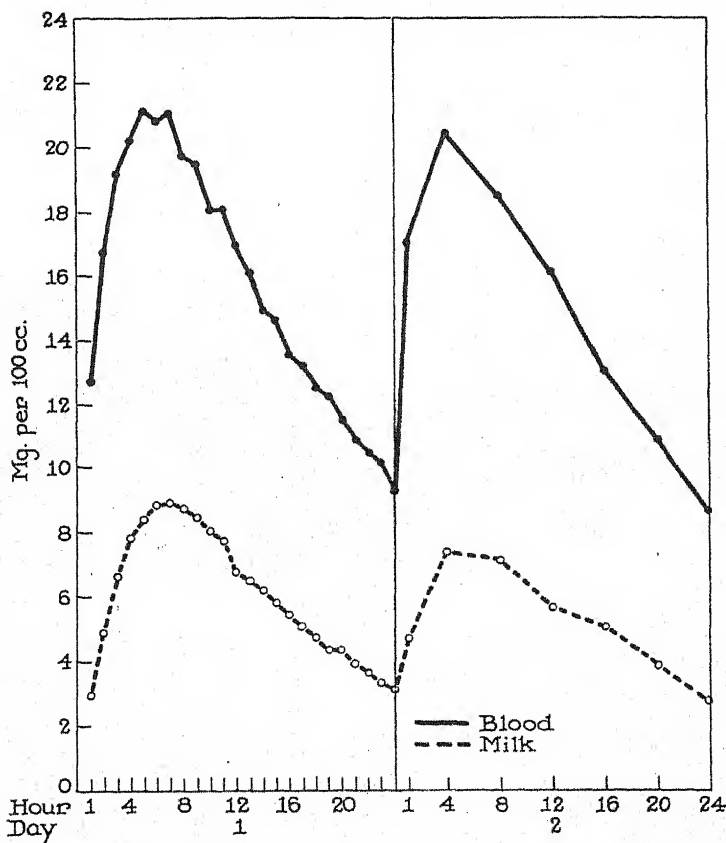


FIG. 1. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day,  $1\frac{1}{2}$  gr./lb. body weight, subcutaneously; 2nd day, 1 gr./lb. body weight, subcutaneously. Milked at 0 and 12 hr.)

dose of sodium sulfamethazine 25 per cent w/v sterile solution was injected, subcutaneously, into each of four cows. (b) Sulfamethazine powder, in 1-ounce capsules, was administered orally, once a day, to two cows. (c) Sodium sulfamethazine 10 per cent w/v sterile solution was infused into the udders of four cows. Half of each total daily dose was administered immediately following complete morning and evening milkings, and equal amounts of each dose were infused into each quarter.



Sulfonamide analyses were made according to a modification (18) of the Bratton-Marshall method (4). Blood and milk samples were taken every hour for the first 24 hours, and at 1 hour, 4, 8, 12, 16, and 24 hours for the second 24 hours. Sulfamethazine determinations in milk were made for each period on each cow, using a composite sample from all quarters. Accurate records of milk production in pounds were kept for 9 days before the start of, as well as for the duration of, the experiment.

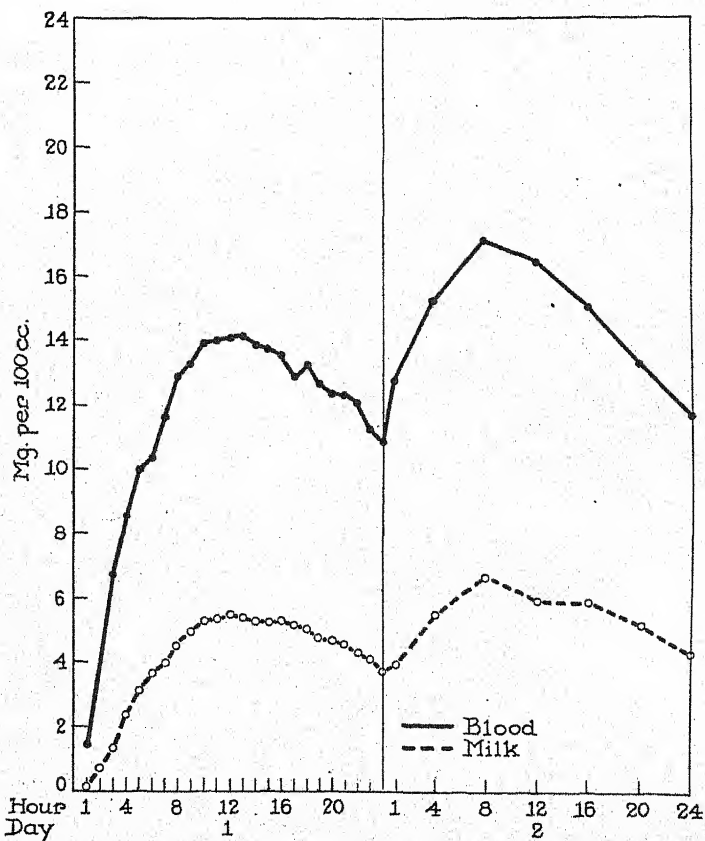


FIG. 2. Free sulfamethazine blood and milk concentrations in cows.  
(Drug administration: 1st day,  $1\frac{1}{2}$  gr./lb. body weight, orally; 2nd day, 1 gr./lb. body weight, orally. Milked at 0 and 12 hr.)

#### RESULTS

Average sulfamethazine blood and milk concentrations following subcutaneous administration are shown in figure 1. High blood levels were attained promptly, reaching a peak at about the fourth hour. The milk level curve closely followed the blood level curve, indicating that the

concentration in the milk is directly dependent on the blood concentration. The milk level was slightly less than half the blood level, which shows that an adequate milk level is dependent on a high blood level.

Figure 2 shows blood and milk concentrations after oral administration. The levels increased more slowly, reaching a peak on the first day between

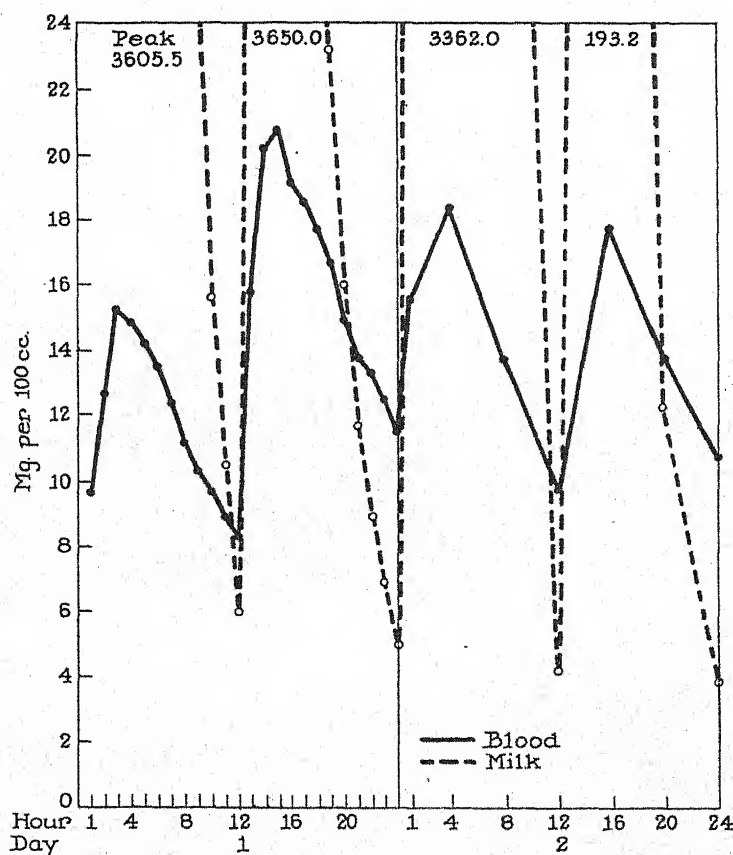


FIG. 3. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day,  $1\frac{1}{2}$  gr./lb. body weight, intramammary infusion; 2nd day, 1 gr./lb. body weight, intramammary infusion. Half of each daily dose was administered immediately following the complete morning and evening milkings, and equal amounts of each dose were infused into each quarter. Milked at 0 and 12 hr.)

the eighth and twelfth hours and, on the second day, at about the eighth hour. The levels attained were not so high as after subcutaneous administration, but, likewise, did not decrease so rapidly. On the second day, the levels attained were higher than on the first day. This would indicate that, with an acutely ill animal, a prompt high blood level should be attained

by parenteral administration and maintained thereafter by oral dosing. Here, too, the milk level curve closely followed the blood level curve.

The concentrations attained by udder infusion are shown in figure 3. As might be expected, milk levels were extremely high after each infusion, rapidly decreasing from the second to the twelfth hour. Blood levels averaging between 10 and 15 mg. per 100 cc. of blood were attained within 3 hours after the first infusion, and gradually decreased to slightly more than 8 mg. per 100 cc. by 12 hours; they were maintained considerably higher after each of the next three infusions. It is evident, therefore, that sulfamethazine diffuses from udder to blood as well as from blood to milk.

Immediately following udder infusion, flakes were observed in the milk. This condition persisted for approximately 4 hours, when the milk again was normal in appearance.

In the normal cow, the decrease in milk production following administration of the drug by any of the routes described was not considered significant. The animals were being handled continuously during the 2-day trial periods, and this, in itself, would affect milk flow. During the three trials, average milk production decreased by 14 per cent, 16.5 per cent, and 18.5 per cent, respectively, from the average daily production during the 9 days preceding the experiment.

#### SUMMARY

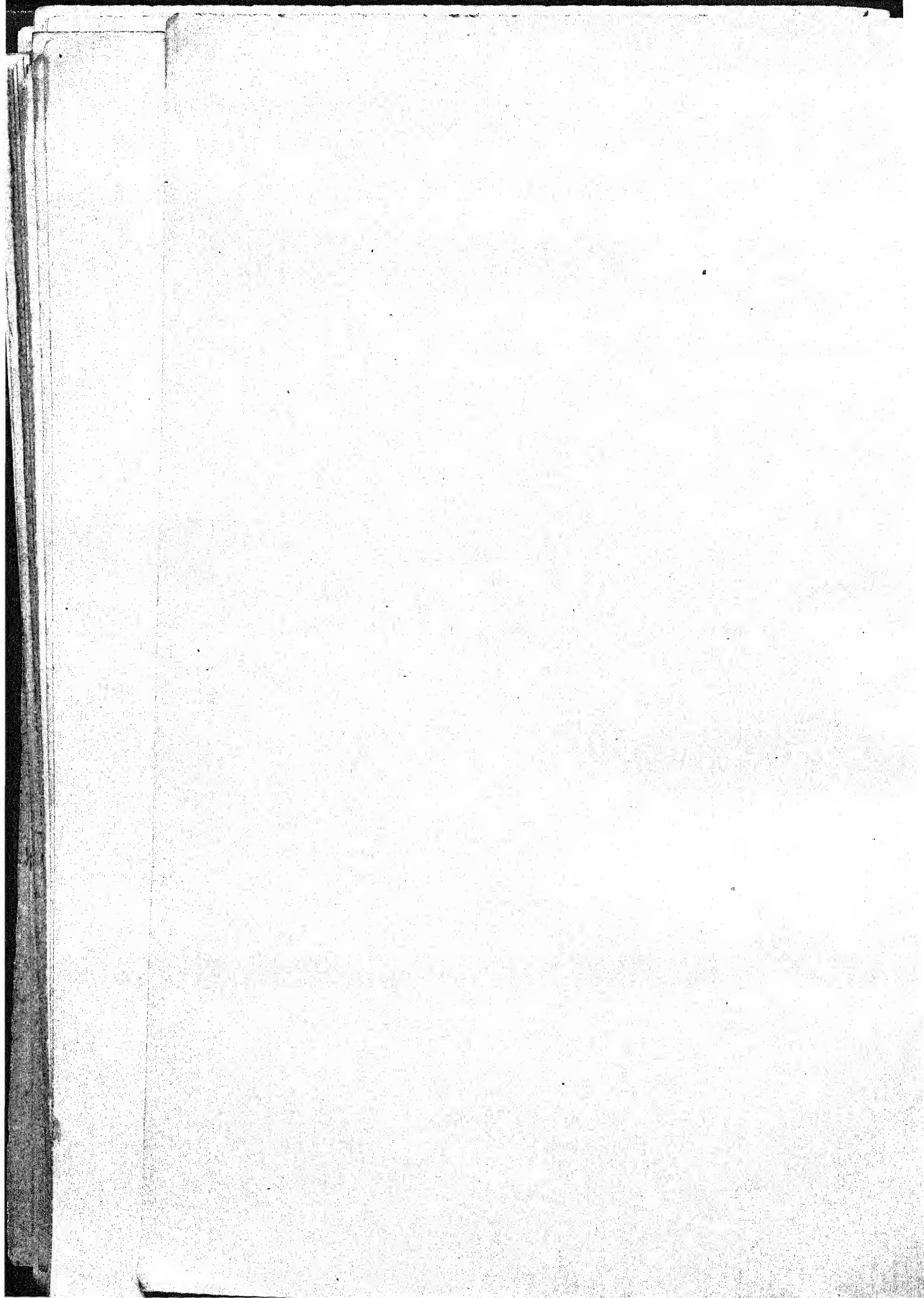
1. Sulfamethazine was administered to cows parenterally, orally, and by infusion, and blood and milk determinations were made at frequent intervals after administration.
2. Sulfamethazine diffuses freely from blood to milk and from udder to blood.
3. Concentrations of 5 mg. or more of the drug per 100 cc. of milk throughout the day depend upon a persistently high concentration (more than 10 mg. per 100 cc.) of the drug in the blood.
4. All three methods of administration resulted in the attainment of blood and milk concentrations considered to be bacteriostatically effective.
5. No evidences of systemic toxicity of the drug were noted.
6. In this experiment, it has been shown that levels above 5 mg. per 100 cc. of milk can be achieved after oral or parenteral administration of the drug given once a day.

#### REFERENCES

- (1) BARKER, C. A. V. Observations on the Use of Penicillin in the Treatment of Bovine Mastitis. *Can. J. Comp. Med. Vet. Sci.*, 9: 235-243. 1945.
- (2) BARKER, C. A. V., AND DUSSAULT, H. P. Observations of Permeability of Lactating Bovine Mammary Gland to Penicillin. *Can. J. Comp. Med. Vet. Sci.*, 9: 332-335. 1945.
- (3) BEAN, C. W., MILLER, W. T., AND HEISHMAN, J. O. Chemotherapy in Mastitis. *Vet. Med.*, 37: 401. 1942.



- (4) BRATTON, A. C., AND MARSHALL, E. K. A New Coupling Component for Sulfanilamide Determination. *J. Biol. Chem.*, 128: 537-550. 1939.
- (5) BRYAN, C. S. The Results Obtained with Tyrothricin in the Treatment of 157 Cows with Streptococcal Mastitis. *Vet. Med.*, 37: 364-369. 1942.
- (6) BRYAN, C. S., HORWOODS, R. E., AND HUFFMAN, C. F. A Preliminary Report on Penicillin in the Treatment of Chronic Streptococcal Mastitis. *Vet. Med.*, 40: 87-89. 1945.
- (7) EDITORIAL. *Hoard's Dairyman*, 92: 638. 1947.
- (8) JOHNSON, S. D., AND ROBERTS, S. J. Combination of Sulfonamide Compounds and Penicillin for the Treatment of Mastitis by Infusion. A Preliminary Report. *Cornell Vet.*, 37: 144-155. 1947.
- (9) KAKAVAS, J. C. Penicillin in the Treatment of Bovine Mastitis. *North Am. Veterinarian*, 25: 408-412. 1944.
- (10) KAKAVAS, J. C. *In vitro* Studies of the Basis for Sulfanilamide Therapy in Bovine Mastitis. *Am. J. Vet. Research*, 6: 9-16. 1945.
- (11) KLEIN, L. A., CRISMAN, D. M., AND MOOR, J. W. Effect of Local Injections of Penicillin on Staphylococci in the Cow's Udder. *Am. J. Vet. Research*, 6: 3-8. 1945.
- (12) LITTLE, R. B., BROWN, J. H., AND PLASTRIDGE, W. N. Bacteriology of Mastitis; in Little & Plastridge: Bovine Mastitis. McGraw-Hill Book Co., Inc., New York, N. Y. Pp. 167-202. 1946.
- (13) SCHALM, O. W. The Treatment of Chronic Bovine Mastitis. *J. Am. Vet. Med. Assoc.*, 100: 323-334. 1942.
- (14) SCHALM, O. W., AND LITTLE, R. B. Treatment of Bovine Mastitis; in Little & Plastridge: Bovine Mastitis. McGraw-Hill Book Co., Inc., New York, N. Y. Pp. 363-416. 1946.
- (15) SEELEY, H. W., JR., ANDERSON, E. O., PLASTRIDGE, W. N., AND PEARSON, P. Non-permeability of the Lactating Bovine Mammary Gland to Penicillin. *Science*, 102: 44-45. 1945.
- (16) SLANETZ, L. W., AND ALLEN, F. E. Penicillin Therapy in Streptococcal Mastitis. *J. Am. Vet. Med. Assoc.*, 111: 125-127. 1947.
- (17) WATTS, P. S., AND MCLEOD, D. H. The Estimation of Penicillin in Blood Serum and Milk of Bovines after Intramuscular Injection. *J. Comp. Path. Therap.*, 56: 170-179. 1946.
- (18) WELSH, M., SCHROEDER, C. R., VROMAN, D. F., REDDIN, L., BURKHART, R. L., AND LANGER, P. H. The Fate of  $\frac{1}{2}$  gr./lb. Body Weight of 7 Sulfonamides in 7 Animal Species. *U. S. Livestock San. Assoc., Proc. 50th Annual Meeting*. Pp. 213-234. 1946.



THE UTILIZATION OF  $\beta$ -CAROTENE, VITAMIN A ALCOHOL,  
AND THE NATURAL ESTER OF VITAMIN A BY  
HOLSTEIN HEIFERS<sup>1,2,3</sup>

R. H. ROSS,<sup>4</sup> C. B. KNODT, AND N. B. GUERRANT

*Departments of Dairy Husbandry and Agricultural and Biological Chemistry,  
Pennsylvania Agricultural Experiment Station, State College*

Vitamin A is believed to be absorbed from the digestive tract in the alcohol form (1). The question has arisen as to whether vitamin A is most efficiently utilized when ingested as the alcohol form, as the natural esters of vitamin A or as  $\beta$ -carotene. A study was conducted to determine the relative efficiency of utilization of  $\beta$ -carotene, vitamin A alcohol, and the natural ester of vitamin A.

EXPERIMENTAL PROCEDURE

Six Holstein heifers between the ages of 12 and 15 months were placed on a low-carotene ration consisting of oat straw fed *ad libitum* and 10 lb. of a concentrate mixture low in carotene. The animals were maintained on this ration until the blood plasma vitamin A decreased to 6-8  $\gamma$  per 100 ml. of blood plasma. They then were grouped into three pairs based upon age, body weight, and blood plasma vitamin A concentration. The three pairs of animals then received in rotation each of the three sources of vitamin A for a period of 20 days at the rate of 100 USP units of vitamin A per kg. of body weight per day. After the first and second feeding periods, the animals again were depleted to 6-8  $\gamma$  of vitamin A per 100 ml. of blood plasma before starting the subsequent supplementary feeding. Thus, after three feeding periods of 20 days each, all six animals had received the three forms of vitamin A. In all instances the vitamin A supplement was administered daily in capsules during the feeding periods.

Blood samples were taken at weekly intervals during the depletion periods and daily during the feeding periods, except in the last half of the first 20-day test period; during this time, they were taken every other day. Blood plasma carotene and vitamin A were determined using the methods of Moore (3) and Kimble (2), respectively, while using an Evelyn photoelectric colorimeter.

Body weights were determined twice a month during the depletion periods and every 3 days during the feeding periods.

Received for publication October 9, 1947.

<sup>1</sup> Authorized for publication on October 2, 1947, as Paper no. 1395 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

<sup>2</sup> The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

<sup>3</sup> This work was supported in part by The Borden Company, New York, N. Y.

<sup>4</sup> Now associated with the University of Idaho, Moscow.



TABLE 1  
The concentrations of blood plasma vitamin A of Holstein heifers fed  $\beta$ -carotene, vitamin A alcohol, and the natural esters of vitamin A<sup>a,b</sup>

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Period of $\beta$ -carotene administration																				
717	5	6	6	5	6	11	11	9	10	9	10	8	7	8	7	8	8	7	7	7
718	13	9	9	11	10	8	10	9	11	10	12	*	8	*	10	*	11	*	9	9
724	15	6	9	7	4	5	4	5	4	5	5	6	4	4	5	10	4	6	7	7
725	4	4	4	4	1	10	7	6	7	9	6	4	5	2	5	5	5	5	5	9
726	11	9	10	6	6	8	8	8	10	*	8	*	6	2	6	7	8	5	8	8
730	10	6	8	7	5	2	1	2	3	2	5	4	4	2	4	5	4	4	2	5
Av.	9.7	6.7	7.7	6.7	5.3	7.3	6.8	6.5	7.5	6.3	7.7	5.5	5.7	4.0	6.2	7.5	6.7	5.5	6.3	7.5
Period of vitamin A alcohol administration																				
717	10	9	9	10	8	8	8	9	10	12	11	12	13	10	11	13	12	10	13	12
718	9	12	15	14	14	17	18	17	20	21	23	15	12	13	14	14	14	13	12	14
724	11	11	12	12	7	8	9	10	12	*	12	*	8	*	13	*	12	*	11	12
725	11	6	8	6	4	4	4	6	4	7	7	7	9	10	7	8	9	7	11	12
726	5	9	8	10	4	12	10	11	12	11	13	11	10	10	11	11	10	12	10	10
730	8	10	9	10	10	12	17	11	14	*	13	*	10	*	11	*	12	*	12	11
Av.	9.0	9.5	10.2	10.3	7.8	10.2	11.0	10.6	12.0	12.8	13.2	11.3	10.3	10.8	11.2	11.5	11.5	10.5	11.5	11.5
Period of administration of the natural esters of vitamin A																				
717	11	14	20	11	7	8	9	11	11	*	12	*	9	*	15	*	15	*	15	16
718	12	11	11	13	11	11	12	11	11	13	13	13	12	11	11	13	12	11	13	14
724	8	10	9	9	7	12	12	11	16	18	13	16	10	11	10	13	11	11	11	13
725	10	9	11	8	8	9	12	10	12	*	11	*	10	*	12	*	13	*	10	10
726	8	7	10	9	7	6	8	7	10	9	10	10	10	8	10	12	9	10	9	9
730	4	7	7	9	8	10	9	11	13	14	14	10	11	10	10	12	12	10	13	11
Av.	8.8	9.7	11.3	9.8	8.0	9.3	10.3	10.2	12.2	13.5	12.2	10.3	10.3	10.0	11.3	12.5	12.0	10.5	11.8	12.2

<sup>a</sup> Fed at the rate of 100 USP units per kilo of body weight per day.

<sup>b</sup> Expressed as  $\gamma$  of vitamin A per 100 ml. of blood plasma.

\* No analysis.

TABLE 2  
The blood plasma concentrations of carotene of heifers fed  $\beta$ -carotene, vitamin A alcohol and the natural esters of vitamin A<sup>a,b</sup>

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Period of $\beta$ -carotene administration																				
717	52	58	56	58	69	67	69	69	82	80	91	94	89	85	85	100	103	94	85	98
718	40	44	46	50	52	50	52	48	48	*	61	*	67	*	78	*	78	*	73	78
724	63	65	76	65	69	80	85	80	76	80	85	91	94	96	96	94	96	96	96	98
725	33	35	37	42	44	50	50	52	58	63	61	58	56	56	59	63	56	61	61	63
726	50	54	58	54	58	56	69	65	65	*	86	*	96	*	86	*	86	*	89	87
730	42	44	54	52	56	58	58	54	54	58	63	65	61	61	63	65	65	65	59	61
Av.	46.7	50.0	54.5	53.5	58.0	60.2	63.8	61.3	63.8	70.3	74.2	77.8	77.5	74.5	77.8	80.5	80.7	79.0	77.2	80.8
Period of administration of vitamin A alcohol																				
717	58	54	54	52	54	54	50	48	44	46	44	50	42	38	42	50	46	42	42	40
718	38	46	42	40	42	40	38	40	40	44	44	37	37	35	35	40	42	40	33	35
724	67	69	65	69	56	54	44	50	46	*	49	*	52	*	56	*	53	*	52	56
725	40	38	42	38	38	35	38	38	37	38	40	44	44	44	38	42	35	37	35	31
726	23	25	25	29	27	23	23	25	27	27	31	29	25	27	27	31	31	33	31	31
730	50	54	50	56	54	50	56	50	52	*	59	*	65	*	56	*	54	*	56	49
Av.	46.0	47.7	46.3	47.3	45.2	42.7	41.5	41.8	41.0	38.8	44.2	39.0	44.2	36.0	42.3	40.8	43.5	38.0	41.5	40.3
Period of administration of the natural esters of vitamin A																				
717	76	78	78	74	78	65	61	67	65	*	70	*	68	*	76	*	75	*	66	67
718	48	54	48	50	48	44	44	44	42	48	46	52	52	46	50	50	50	48	48	44
724	67	67	63	63	67	61	59	58	61	69	56	65	54	54	52	59	54	54	58	59
725	42	42	42	42	38	37	35	35	33	*	43	*	48	*	42	*	46	*	42	39
726	50	50	56	50	48	48	46	40	44	48	50	48	48	46	46	46	46	46	42	38
730	33	33	31	29	29	27	27	27	29	31	31	27	25	38	25	27	29	27	31	29
Av.	52.7	55.0	53.0	51.3	51.3	47.0	45.3	45.2	45.7	49.0	49.3	48.0	49.2	46.0	48.5	45.5	50.0	43.8	47.8	46.0

<sup>a</sup> Fed at the rate of 100 USP units per kilo of body weight per day.

<sup>b</sup> Expressed as  $\gamma$  of carotene per 100 ml.

\* No analyses made on first trial on these days.

## RESULTS

Vitamin A values of 6 to 8  $\gamma$  per 100 ml. of blood plasma appeared to be the critical level for these animals. When the concentration of vitamin A approached this level, the test animals stopped gaining in body weight. However, the animals usually resumed growth after about 10 days of supplemental feeding. The average time required to deplete the animals was 105 days following winter feeding, 24 days between the first and second feeding periods, and 30 days between the second and third feeding periods.

The data obtained relative to the analyses for vitamin A and carotene on the several trials are presented in tables 1 and 2.

In evaluating the blood plasma vitamin A data for the three supplementary treatments by an analysis of variance (table 3), a highly significant

TABLE 3  
*Analysis of variance of blood plasma vitamin A data*

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total .....	179	2261	
Treatments .....	2	593	296.50 <sup>a</sup>
Individuals .....	5	393	78.60 <sup>b</sup>
Days .....	9	189	21.00 <sup>a</sup>
Interactions:			
Treatments $\times$ individuals .....	10	242	24.20 <sup>a</sup>
Days $\times$ individuals .....	45	137	3.04
Days $\times$ treatments .....	18	134	7.44
Sampling error .....	90	573	6.37

<sup>a</sup> Significant at the 1% level.

<sup>b</sup> Approached significance at the 5% level.

difference was found between the treatments and between days of supplementation. The difference between heifers approached significance. On the basis of the least significant mean difference, it was determined that there was no significant difference in the blood plasma vitamin A concentrations of the heifers during administration of vitamin A alcohol or the natural esters of vitamin A. Both, however, produced a higher level (highly significant) of blood plasma vitamin A than did  $\beta$ -carotene. The following mean concentrations of blood plasma vitamin A ( $\gamma$  per 100 ml.) were found during the feeding periods:  $\beta$ -carotene feeding = 6.95, vitamin A alcohol feeding = 10.77, and the natural ester of vitamin A feeding = 10.83. The least significant mean difference at the 1 per cent level was 2.85.

In an analysis of variance of the determinations of blood plasma carotene of the heifers for the three treatments (table 4), a highly significant difference was found between treatments and between days on supplementation, but no significant difference was found between heifers. The following mean concentrations of blood plasma carotene ( $\gamma$  per 100 ml.) were determined



TABLE 4  
Analysis of variance of blood plasma carotene data

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total .....	179	54,826	
Treatments .....	2	18,607	9,303.50 <sup>a</sup>
Individuals .....	5	13,997	2,799.40
Days .....	9	1,909	212.11 <sup>a</sup>
Interactions:			
Treatments × individuals ..	10	10,837	1,083.70 <sup>a</sup>
Days × individuals .....	45	839	18.64
Days × treatments .....	18	6,089	338.28 <sup>a</sup>
Sampling error .....	90	2,548	28.31

<sup>a</sup> Significant at the 1% level.

during the feeding periods:  $\beta$ -carotene administration = 67.42, vitamin A alcohol administration = 43.57, and the natural ester of vitamin A = 49.28. The least significant mean difference at the 1 per cent level was 19.05. On this basis it was found that the feeding of  $\beta$ -carotene increased the blood plasma carotene concentration above (highly significant) that of the heifers receiving vitamin A. There was no significant difference in the blood plasma carotene concentrations when vitamin A alcohol or the natural ester of vitamin A was fed.

Linear regression lines were calculated for blood plasma vitamin A (Fig. 1) and carotene (Fig. 2) concentrations during the feeding periods. The regression equation for blood plasma vitamin A concentration when  $\beta$ -carotene was fed was  $E = 7.422 - 0.0692 X \pm 0.0442$  (not significant); when the natural ester of vitamin A was fed,  $E = 9.403 + 0.1412 X \pm 0.0384$

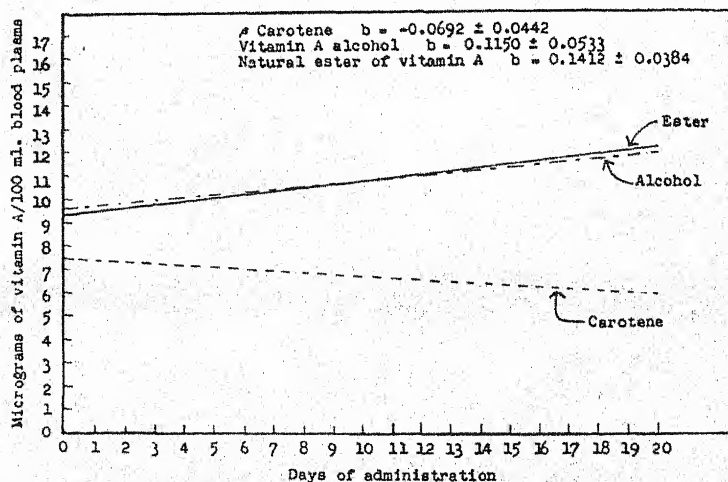


FIG. 1. The effect of the source of vitamin A upon blood plasma vitamin A levels.

(highly significant); and when the vitamin A alcohol was fed,  $E = 9.61 - 0.1150 X \pm 0.0533$  (significant). Therefore, there was a significant increase in blood plasma vitamin A concentration when vitamin A alcohol or the natural esters of vitamin A were fed, and there was a slight decrease when  $\beta$ -carotene was fed. This decrease, however, was not significant.

Regression equations for blood plasma carotene concentrations are as follows: when  $\beta$ -carotene was fed,  $E = 48.70 + 1.8325 X \pm 0.2186$  (highly significant); when the natural ester of vitamin A was fed,  $E = 51.62 - 0.2934 X \pm 0.2211$ ; and when vitamin A alcohol was fed,  $E = 46.45 - 0.3313 X \pm$

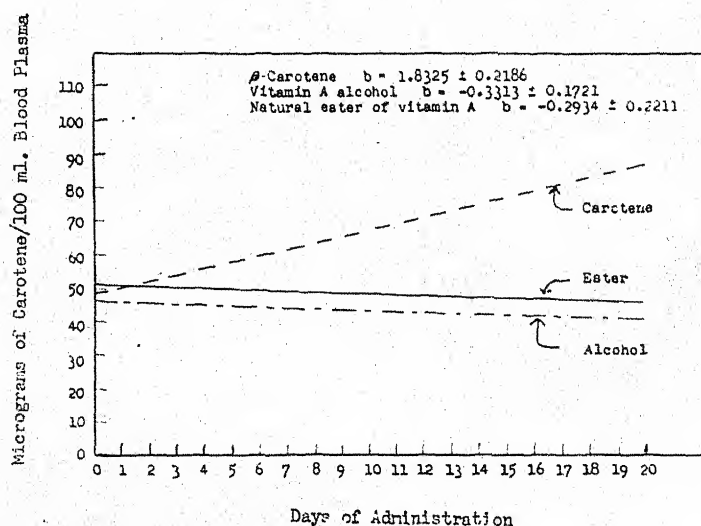


Fig. 2. The effect of the source of vitamin A upon blood plasma carotene levels.

0.1721. Therefore, feeding  $\beta$ -carotene increased the blood plasma carotene concentration significantly, but, when either of the two forms of vitamin A was fed, the blood plasma carotene concentration decreased slightly. This decrease, however, was not statistically significant.

#### CONCLUSIONS

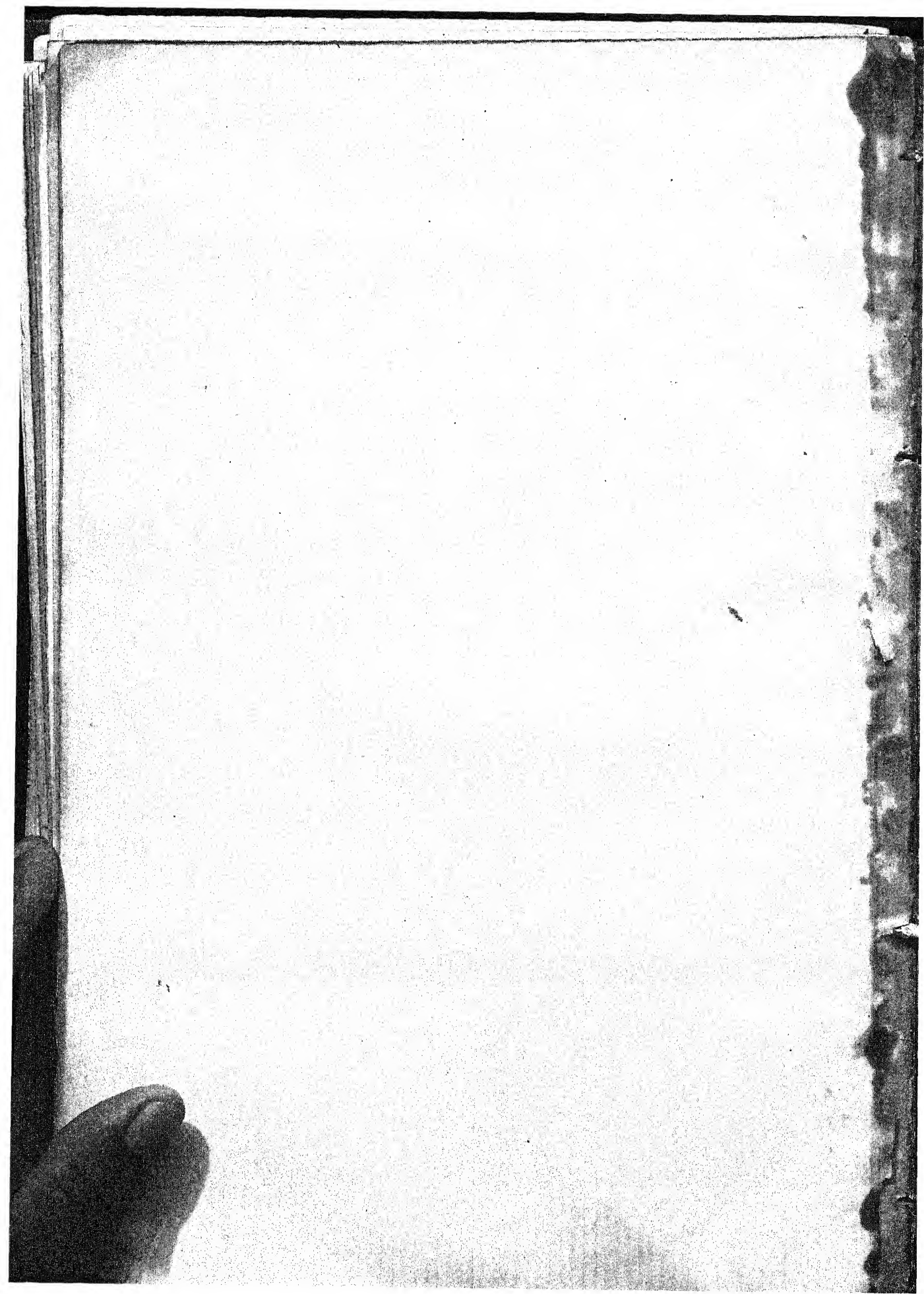
1. The critical blood plasma vitamin A concentration was found to be 6 to 8  $\gamma$  per 100 ml. blood plasma for Holstein heifers when gains in body weight were used as the criterion.

2. There was no significant difference in the efficiency of utilization of vitamin A alcohol and the natural esters of vitamin A by Holstein heifers when the blood plasma concentration of vitamin A was used as a criterion. However, both forms of vitamin A were utilized more efficiently than  $\beta$ -carotene.

## REFERENCES

- (1) GRAY, E. L., MORGAREIDGE, K., AND CAWLEY, J. D. Intestinal Absorption of Vitamin A in the Normal Rat. *J. Nutrition*, 20(1): 67-74. 1940.
- (2) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, 24: 1055-1065. 1939.
- (3) MOORE, L. A. The Determination of Blood Plasma Carotene in the Bovine Using a Photoelectric Colorimeter. *J. Dairy Sci.*, 22(7): 501-511. 1939.





## LIVEWEIGHT AND MILK-ENERGY YIELD AT VARIOUS FEEDING INTENSITIES<sup>1</sup>

S. D. MUSGRAVE<sup>2</sup> AND W. L. GAINES

*University of Illinois, Urbana*

The data used in the present paper are extracted from the records of the Input-Output experiment conducted by the United States Bureau of Dairy Industry in cooperation with the Agricultural Experiment Stations of Delaware, Maryland, Mississippi, New York (Geneva), Pennsylvania, South Dakota, Indiana, Michigan, New Jersey, and Virginia. In the last four of these Stations, pasture was used, thereby upsetting the determination of digestible nutrients (D.N.) intake. A primary objective in the use made of the Bureau data at the Illinois Station is to allocate D.N. intake between maintenance and lactation by the procedure of fitting a suitable equation, for which purpose only the first six of the above-mentioned stations provide adequate data. Pursuit of the primary objective has provided, somewhat incidentally, valuable material on the relation between liveweight and milk-energy yield, which is reported in the present paper.

<sup>1</sup>A detailed account of the Input-Output investigation has been published by Jensen *et al.* (2).

### PROCEDURE

The first 35 full calendar weeks of each lactation are used in the present study. Lactations which do not provide such a period are not used. That is, the present paper deals with partial lactations, starting within 9 days after calving (the first 2 days after calving being rejected in the original records) and continuing through the following 35 calendar weeks. The records provide a total of 255 such partial lactations.

Each 35-week partial lactation is extracted in seven subperiods of 5 weeks each, and the sum of seven subperiods represents the 35-week partial lactation. For each lactation the following items, among others, are calculated:

$D.N.$  = digestible nutrient intake for 35-week period, lb./day

$FCM_s$  = milk-energy yield for 35-week period, lb. 4 per cent milk/day

$W$  = average liveweight for 35-week period, lb.

$W_1$  = average liveweight for first 5-week subperiod, lb.

Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds are repre-

Received for publication October 20, 1947.

<sup>1</sup> The authors are indebted to O. E. Reed, Chief of the Bureau of Dairy Industry, for administrative approval of the present use of the records of the Input-Output investigation; also, to T. E. Woodward, Bureau of Dairy Industry, for painstaking care in providing photostatic copies of the weekly records maintained by the Bureau in conduct of the original investigation.

<sup>2</sup> Now at Cornell University, Ithaca, New York.

sented in the present material (table 1). Perhaps the cows represent a cross section of dairy cows in American Experiment Stations.

#### RESULTS

*Feeding intensity.* Feeding intensity is calculated as  $(D.N. - 0.008W)/FCM_s$ , i.e., lb. D.N. for lactation per lb.  $FCM_s$ , allowing 8 lb. D.N. per day per 1,000 lb. liveweight for maintenance, in accord with the Haecker standard. The data are divided into feeding intensity classes as indicated in table 2. These classes are based on the actual rather than the intended feed intake. Consequently, the classes do not correspond strictly with the classes used by Jensen *et al.* (2), and there is the further important difference that the present analysis is based on a 35-week partial lactation, in contrast to a calendar-year record regardless of lactation status.

TABLE 1  
The number of lactations and mean values by breeds

Breed	n	% Haecker	% Grain <sup>a</sup>	$W_1$	$1000 FCM_s/W_1$
Ayrshire .....	29	120	35	1035	25.7
Brown Swiss .....	25	107	52	1228	31.8
Guernsey .....	21	114	31	998	24.6
Holstein .....	104	114	46	1204	31.1
Jersey .....	76	123	45	844	31.0

<sup>a</sup> Per cent of digestible nutrient intake supplied by concentrates.

*Liveweight and yield.* It previously has been proposed that cows possess a certain inherent tendency to produce milk under conditions of the commercial dairy, a lactational drive which may be quantitatively measured as  $FCM_s/W_1$ . The immediate purpose of the present study is to see how  $FCM_s/W_1$  is affected by feeding intensity.

In the light of previous work the postulate is advanced that  $FCM_s/W_1$  fluctuates, as between cows, independently of  $W_1$ . A proper test of this postulate is to fit, by least squares, the equation,  $FCM_s/W_1 = a + bW_1$  and find the value of  $b$  and its standard error. For the 255 lactations as a body, as shown in the last line and last column of table 2,  $b$  is even smaller than its standard error. Such a value of  $b$  readily could arise by chance if its true value is zero. Hence, the postulate is valid so far as this particular body of observations indicates.

By a similar equational procedure the value of  $b$  (in terms of 100,000  $FCM_s/W_1$ ) within feeding intensity class works out to be  $0.21 \pm 0.23$  or essentially the same result as that in total.<sup>3</sup>

If it is desired to use the power equation  $FCM_s = aW_1^b$ , the exponent  $b$  is 0.92 in total and 0.93 within feeding-intensity class. The exponent is derived from the means and linear regression. For example, in total, the ex-

<sup>3</sup> In similar manner: within Station,  $b = -0.109 \pm 0.036$ ; within breed,  $b = -0.109 \pm 0.039$ ; within times milked daily (2 or 3),  $b = -0.061 \pm 0.024$ .



TABLE 2  
Feeding intensity and milk-energy yield per unit liveweight

Feeding intensity					Lactations	Mean		Value of <i>b</i> in the equation	
Class	Class limit	Mean	% Haecker	% grains		<i>W</i> <sub>1</sub>	1000 <i>FCM</i> <sub>s</sub> / <i>W</i> <sub>1</sub>	<i>FCM</i> <sub>s</sub> = <i>aW</i> <sub>1</sub> <sup>b</sup>	100000 <i>FCM</i> <sub>s</sub> / <i>W</i> <sub>1</sub> = <i>a</i> + <i>bW</i> <sub>1</sub>
1	< 0.30	0.273	83	28	35	1074	26.2	+ 1.34	+ 0.83 ± 0.67
2	0.30	0.327	100	36	59	1078	27.3	+ 1.04	+ 0.10 ± 0.43
3	0.35	0.374	114	47	71	1088	32.0	+ 0.99	- 0.04 ± 0.50
4	0.40	0.422	129	52	54	1047	32.8	+ 0.95	- 0.17 ± 0.45
5	0.45	0.474	145	53	20	1031	28.6	+ 0.92	- 0.21 ± 0.94
6	0.50	0.600	183	53	16	968	30.3	- 0.07	- 3.34 ± 0.96
All		0.381	117	44	255	1063	30.0	+ 0.92	- 0.22 ± 0.25

<sup>a</sup> Per cent of digestible nutrient intake supplied by concentrates.

ponent =  $1 + (-0.22 \times 1063/3000) = 1 - 0.08 = 0.92$ . This is a valid approximation for the present material.

While the above procedure indicates no difference in total and within feeding-intensity class, it does not necessarily follow that feeding intensity is without influence on the relation of liveweight to milk-energy yield. Table 2 shows the weight-yield relation for each of the six classes separately. None of the  $b$ 's is significant except the one for the 16 lactations of class 6. The  $W_1$  distribution in class 6 is erratic and apparently the cows were more ravenous than representative.

A noteworthy feature is the rather consistent decrease in  $b$  as feeding intensity increases. The power-equation  $b$  shows this clearly. The data seem to suggest that  $FCM_s$  tends to be proportional to  $W_1$  under customary feeding intensity (100 per cent or 114 per cent of Haecker standard for the lactation fraction).  $W_1$  appears still more influential on  $FCM_s$  for under-feeding, which may trace back to the influence of fatness at calving.

#### DISCUSSION

The average of 255 35-week partial lactations (table 2) is 30.0 lb. of 4 per cent milk per day per 1,000 lb.  $W_1$ . Davis *et al.* (1) report for the Nebraska Station dairy herd average values for 1000  $FCM_s/W_1$  of 33.3 for 131 Ayrshire lactations, of 30.6 for 77 Guernsey lactations, of 39.1 for 367 Holstein lactations and of 34.5 for 171 Jersey lactations. Feeding intensity for the Nebraska data is not recorded but no doubt is above 100 per cent Haecker for all lactations. Control of feeding intensity was a major point in the Input-Output experiment. It is presumed this control does not bias the results as between breeds.

#### SUMMARY

The relation of liveweight in pounds within 5 weeks after calving,  $W_1$ , to milk-energy yield for the 35-week partial lactation in pounds of 4 per cent milk per day,  $FCM_s$ , is investigated by adjusting the equation,  $FCM_s/W_1 = a + bW_1$ , to observations from the Input-Output experiment of the Bureau of Dairy Industry. For all lactations (255),  $b$  is not significantly different from zero. The postulate that  $FCM_s$  tends to be proportional to  $W_1$  is valid so far as indicated by this body of observations on five breeds of dairy cows taken as a whole. Essentially the same relation holds for each feeding-intensity class (83 to 183 per cent of Haecker). However, as between feeding-intensity classes, there is a consistent tendency for  $b$  to decrease as feeding intensity increases. The consistency of this tendency may give it some meaning.

#### REFERENCES

- (1) DAVIS, H. P., MORGAN, R. F., AND GAINES, W. L. Live Weight and Milk-Energy Yield in the Nebraska Station Dairy Herd. *J. Dairy Sci.*, 26: 625-641. 1943.
- (2) JENSEN, E., KLEIN, J. W., RAUCHENSTEIN, E., WOODWARD, T. E., AND SMITH, R. H. U. S. Dept. Agr. Tech. Bull. 815. 1942.

## THE CHEMICAL COMPOSITION OF THE CRYSTALLINE DEPOSIT IN EVAPORATED MILK

EDGAR F. DEYSHER AND B. H. WEBB

*Bureau of Dairy Industry, Agricultural Research Administration,  
U. S. Department of Agriculture*

A white, crystalline deposit occasionally forms in evaporated milk during storage. It is found chiefly on the interior can surfaces but as the crystals increase in size and weight, agitation of the contents of the can gradually causes them to settle. The deposit does not appear in some samples of milk and, even when it is present, it remains unnoticed by most consumers. While the crystals are not harmful, they are at times a source of annoyance, especially in infant feeding when they obstruct the holes in nipples. Occasionally the crystal aggregates are mistaken for foreign particles.

The deposit cannot be redissolved in the milk after it has formed. The particles themselves vary from microscopic size to crystal aggregates  $\frac{3}{8}$ -inch in diameter. They are dense and hard and should not be confused with the insoluble protein deposit which sometimes is found in evaporated milk.

Sato was the first to investigate the salt crystals of concentrated milk. On the basis of his determinations of calcium, magnesium, and phosphorus in salt crystals found in sweetened condensed milk (9, 11), he reported calcium citrate to be the chief constituent of these crystals. Only qualitative determinations for citrates were reported. Crystals of tyrosin, leucin, and cysteine also were found. Later Sato (10) reported on the examination of the sediment obtained from one 2-year-old can of evaporated milk. A quantitative analysis of this deposit was stated to show that it contained tricalcium and trimagnesium phosphates and tricalcium citrate. The quantitative data and the relative proportions of these salts in the evaporated milk were not given.

Mojonnier and Troy (7) condensed unheated skim milk over sulfuric acid to one-third its volume, stored it at 85° F. for 3 months, and then found a considerable quantity of calcium citrate had crystallized in the milk.

While this manuscript was in preparation, Gould and Leininger (3) published the results of quantitative determinations made in duplicate on a composite sample of crystals separated from evaporated milk. They found that the crystals were largely composed of calcium citrate.

This study of the crystalline deposit that forms in evaporated milk was conducted to determine quantitatively the composition of the crystals and to define the conditions that favor and retard their formation. This paper is concerned with quantitative determinations.

Received for publication October 27, 1947.



## EXPERIMENTAL PROCEDURE

Crystals were obtained from several hundred cans of commercial evaporated milk. The sediment from each can was washed with 25 per cent alcohol, thoroughly agitated, and decanted a number of times until the wash alcohol showed no turbidity. The crystals then were assumed to be reasonably free from foreign matter. They were air dried at room temperature, ground in a mortar, redried at a low temperature under vacuum, and preserved in glass-stoppered bottles for analysis.

The method of McCrudden (4, 5) was used for the calcium determinations. The calcium oxalate precipitate was ignited and weighed as calcium oxide. Magnesium (4, 5) was determined on the filtrate from the calcium analyses. Nitric acid was added to this filtrate and the solution evaporated to dryness to expel the ammonium salts. The residue then was dissolved in a little hydrochloric acid. The usual procedure was carried out and the precipitate was weighed as magnesium pyrophosphate.

The official gravimetric method (1) was used for the determination of phosphorus.

Citric acid was determined by the pentabromoacetone method as modified by Deysher and Holm (2), with a few additional changes. The procedure is outlined here in some detail because citric acid determinations often have been found difficult to make and because good results were obtained with this modification.

Five-tenths gram of the material is dissolved in 40 ml. of sulfuric acid (1 to 1 by volume) in a 250-ml. volumetric flask and several milliliters of 10 per cent phosphotungstic acid are added to precipitate the small amount of protein material. The contents of the flask are made up to 250 ml., thoroughly shaken, and filtered. Fifty milliliters, which is equivalent to 0.1 g. of the sample, are taken for the determination. Five milliliters of 37.5 per cent potassium bromide are added, followed by 5 per cent potassium permanganate added dropwise until a brown precipitate remains for at least an hour. The mixture then is placed in a refrigerator over night, after which the excess potassium permanganate is discharged with 20 per cent ferrous sulfate. After filtering, drying in the vacuum desiccator, and weighing, the precipitate is dissolved with alcohol and ether. The crucible again is dried and weighed. The loss in weight represents the pentabromoacetone which, multiplied by 0.424, is equal to anhydrous citric acid.

This method was tried on C.P. calcium citrate in which the water of crystallization had been determined. The recovery on four determinations was 98.7, 101.7, 100.3, and 99.4 per cent.

## RESULTS

The analytical results obtained on four groups of crystals gathered from cans of commercial evaporated milk are given in table 1.

TABLE 1  
*Analysis of crystals from 4 brands of evaporated milk<sup>a</sup>*

Milk no.	Loss on ignition	CaO	MgO	P <sub>2</sub> O <sub>5</sub>	Anhydrous citric acid	Loss on ignition + CaO + MgO + P <sub>2</sub> O <sub>5</sub>
	(%)	(%)	(%)	(%)	(%)	(%)
1 <sup>b</sup>	70.79	29.0	0.23	0.19	64.25 <sup>c</sup>	100.21
2 <sup>b</sup>	70.40	29.22	0.12	0.44	63.97	100.18
3	65.80	29.90	0.12	4.43	60.71	100.25
4 <sup>b</sup>	70.95	29.10	0.31	0.19	63.60 <sup>c</sup>	100.55

<sup>a</sup> Each group of crystals was collected from cans of milk produced in a single plant and processed on the same or on consecutive days.

<sup>b</sup> Only a trace (0.02%) of SiO<sub>2</sub> was found in a composite of these samples.

<sup>c</sup> Triplicate determinations; all others made in duplicate.

The calcium oxide was found to be about 29.0 per cent and the anhydrous citric acid 63.0 per cent. The composition of C.P. calcium citrate in terms of calcium oxide and anhydrous citric acid is 29.48 per cent and 67.33 per cent, respectively (6).

There was a 9 to 10 per cent loss in weight of the evaporated milk crystals at 120° C., which indicates that water of crystallization was present. According to Merck's Index (6) all the water of crystallization of calcium citrate is lost at 120° C.

The amounts of magnesium oxide in the different samples were fairly uniform but the quantity of phosphorus pentoxide in no. 3 was in large excess over the phosphorus pentoxide in the other three samples. This indicates the presence of a substantial quantity of calcium phosphate in no. 3.

Since the calcium in the calcium caseinate-calcium phosphate complex (8) of milk exists as tribasic phosphate, calculations were made to determine the quantities of tribasic magnesium and calcium phosphate and of calcium citrate that might be present in the crystals. Results of the calculations are presented in table 2. All the magnesium oxide was converted to trimagnesium phosphate, but this required more phosphorus pentoxide than was present in samples 1 and 4, giving the latter a negative phosphate balance.

TABLE 2  
*Calculated values<sup>a</sup> for calcium and magnesium phosphates and for calcium citrate in the salt crystals of evaporated milk*

Milk no.	MgO as Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	P <sub>2</sub> O <sub>5</sub> balance	Remaining P <sub>2</sub> O <sub>5</sub> as Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	CaO balance	Remaining CaO as CaCit. 4H <sub>2</sub> O	Total Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> + Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> + CaCit.4H <sub>2</sub> O
	(%)	(%)	(%)	(%)	(%)	(%)
1	0.50	-0.08	.....	29.00	98.33	98.83
2	0.26	+0.30	0.65	28.87	97.90	98.81
3	0.26	+4.29	9.37	24.82	84.18	93.81
4	0.67	-0.17	.....	29.10	98.68	99.35

<sup>a</sup> Calculated from the determined values shown in table 1.

The remaining phosphorus pentoxide in samples 2 and 3 was converted to tricalcium phosphate. The calcium balance remaining after the phosphorus pentoxide was exhausted was converted to calcium citrate. This required a little more citrate than was found in the samples. The sums of these calculated values for the phosphates and citrates are given in the last column of table 2. With the exception of sample 3, they are not far from 100 per cent.

There are other salt combinations that might be assumed to exist for the purpose of calculating the approximate composition of the crystals. However, in the absence of accurate data on the salt crystal structures, any determination of the manner in which the various components are combined must be deferred.

#### SUMMARY

Four groups of salt crystals that had separated from evaporated milk during storage were analyzed for calcium oxide, magnesium oxide, phosphorus pentoxide and citric acid. One group of crystals was high enough in phosphorus pentoxide to indicate the presence of almost 10 per cent tricalcium phosphate. Most of the crystals contained about 98 per cent calcium citrate and small but varying amounts of tricalcium and trimagnesium phosphates.

#### REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 4th Ed. Washington, D. C. P. 19. 1935.
- (2) DEYSHER, E. F., AND HOLM, G. E. Determination of Citric Acid. Ind. Eng. Chem., Anal. Ed., 14: 4-7. 1942.
- (3) GOULD, I. A., AND LEININGER, E. The Composition of the Solids which Deposit from Evaporated Milk during Storage. Mich. Agr. Expt. Sta. Quart. Bull. 30. Pp. 54-56. 1947.
- (4) MCCRUDDEN, F. H. The Quantitative Separation of Calcium and Magnesium in the Presence of Phosphates and Small Amounts of Iron Devised Especially for the Analysis of Foods, Urine, and Feces. J. Biol. Chem., 7: 83-100. 1909.
- (5) MCCRUDDEN, F. H. The Determination of Calcium in the Presence of Magnesium and Phosphates. J. Biol. Chem., 10: 187-199. 1911.
- (6) MERCK INDEX. Pub. by Merck and Co., Rahway, N. J. 5th Ed. P. 108. 1940.
- (7) MOJONNIER, T., AND TROY, H. C. The Technical Control of Dairy Products. 1st Ed. Mojonnier Bros. Co., Chicago, Ill. P. 762. 1922.
- (8) RAMSDALL, G. A., AND WHITTIER, E. O. Composition of Casein in Milk. J. Biol. Chem., 154: 413-419. 1944.
- (9) SATO, M. Untersuchungen Über Die Milchkristalle in Kondensierter Milch Mit Zuckerzusatz. J. Coll. Agr., Tokyo Imp. Univ., 5: 321-338. 1913-1914.
- (10) SATO, M. Sediments of Evaporated Milk. Proc. World's Dairy Congr., 2: 1284-1285. 1923.
- (11) SATO, M. The Crystals Found in Sweetened Condensed Milk. Proc. World's Dairy Congr., 2: 1285. 1923.



# THE ISOLATION AND PROPERTIES OF THE IMMUNE PROTEINS OF BOVINE MILK AND COLOSTRUM AND THEIR ROLE IN IMMUNITY: A REVIEW

EMIL L. SMITH<sup>1</sup>

*Laboratory for the Study of Hereditary and Metabolic Disorders,<sup>2</sup> and the Departments  
of Biochemistry and Medicine, University of Utah School of Medicine, Salt Lake City*

In mammalian life, the mother supplies the fetus and the newborn offspring with all of the substances necessary for growth. However, in addition to providing the usual food factors, the offspring is given the antibodies necessary to resist infectious diseases. Since the work of Ehrlich (8), it has been known that antibodies may be transmitted through the colostrum or first milk, passively immunizing the offspring by means of immune bodies which are ingested by mouth and then pass from the digestive tract to the blood stream. In addition, Ehrlich discovered that, in some species, immune bodies also may be transmitted through the placenta directly from the blood stream of the mother to the circulation of the fetus.

In the ruminants, placental transmission does not occur, and the colostrum is the sole source of antibodies for the newborn animal (9, 13, 22). Some years ago, Smith and Little (32, 33) investigated the factors concerned with the survival of newborn calves and found that intestinal infections were among the major causes of death. Feeding of colostrum was found to decrease the mortality enormously. Obviously, the antibodies transmitted by the colostrum are of great importance in enabling the newborn animal to resist infection. At about the same time, Howe (10) and Orcutt and Howe (17) observed that, after the ingestion of colostrum, agglutinins appear in the calf serum associated with a globulin which is precipitable at low concentrations of sodium sulfate. Other investigators (11, 21) have since found by electrophoretic analysis that the serum of the newborn calf does not possess any  $\gamma$ -globulin and that the appearance of slow-moving globulin occurs only after the ingestion of colostrum.

In recent years it has been amply demonstrated by many investigators that antibodies are associated with globulin components of the serum, and much has been learned regarding their properties (12). Until recently, no attempts were made to isolate and study the proteins associated with immunity from milk or colostrum.

The immune proteins of bovine colostrum and milk have been isolated in order to determine their relationship to the immune proteins found in blood serum (24, 25, 26). It obviously is of some importance to ascertain

<sup>1</sup> The experimental work included in this paper was performed while the author was at the Biological Laboratories of E. R. Squibb and Sons.

<sup>2</sup> This laboratory is aided by a grant from the United States Public Health Service.

whether the immune bodies in the colostrum and milk are identical or similar to those in the maternal blood stream. It also is of interest to determine what happens to the colostrum antibodies during their passage into the blood stream of the newborn animal. The cow represents a good species for such a study because, in addition to the availability of milk and colostrum, the situation is not complicated by placental transmission of antibodies. It is convenient to refer to the colostrum and milk globulins which are associated with immunity as "immune lactoglobulins", although it is realized that the actual antibody content may account for only a very small portion of these fractions.

#### BOVINE IMMUNE PROTEINS

Colostrum obtained within a few hours after parturition was found to have a protein concentration between 15 and 26 per cent or, roughly, two to three times the concentration of blood plasma (25). By electrophoretic analysis, the immune protein may represent as much as 50 to 60 per cent of the total protein in colostrum, and as high as 85 to 90 per cent of the protein in colostrum whey. Therefore, it was a comparatively simple matter to isolate the immune protein in electrophoretically homogeneous form. After removal of the casein (Fraction *A*) by isoelectric precipitation at pH 4.5, the filtrate was brought to pH 6.0 with 0.5 M sodium hydroxide, and successive fractions were removed at 0.3 (Fraction *B*), 0.5 (Fraction *C*), and 0.9 (Fraction *D*) saturation with ammonium sulfate. After reprecipitation of each fraction within the same limits of salt concentration, the preparations were dialyzed and dried from the frozen state. Figure 1 shows the electrophoretic patterns obtained with the four fractions and with the original colostrum.

The electrophoretic pattern of the whole colostrum shows only a few components, with the slow-moving large peak due to the immune protein. The crude casein of colostrum (Fraction *A*) is complex in nature, like that of milk (15, 35), and contains at least two components (25). Fraction *B* consists entirely of a slow-moving globulin, and Fraction *C* of about 85 per cent of this protein. All of the immune activity of the colostrum was found to be associated with the lactoglobulin of low electrophoretic mobility ( $-1.8$  to  $-2.2 \times 10^{-5}$  sq. cm. per volt per second at pH 8.4).

The lactalbumin (Fraction *D*) is complex in nature and, like the similar fraction of milk whey, consists mainly of the  $\beta$ -lactoglobulin isolated by Palmer (18). The crystalline  $\beta$ -lactoglobulin of colostrum, as far as could be determined, is identical with that obtained from milk (25).

Quantitative isolation of the immune lactoglobulins was accomplished by precipitation at 0.4 saturation with ammonium sulfate after isoelectric precipitation of the casein. After this fraction was reprecipitated several

times under the same conditions, the material was homogeneous. Prolonged dialysis of the protein resulted in a separation of water-insoluble and water-soluble portions or eu- and pseudoglobulin fractions.

Figure 2 shows the electrophoretic patterns obtained with the normal whey of the later milk. Here the immune globulin represents only about 10 per cent of the whey protein in the normal animal, although the pro-

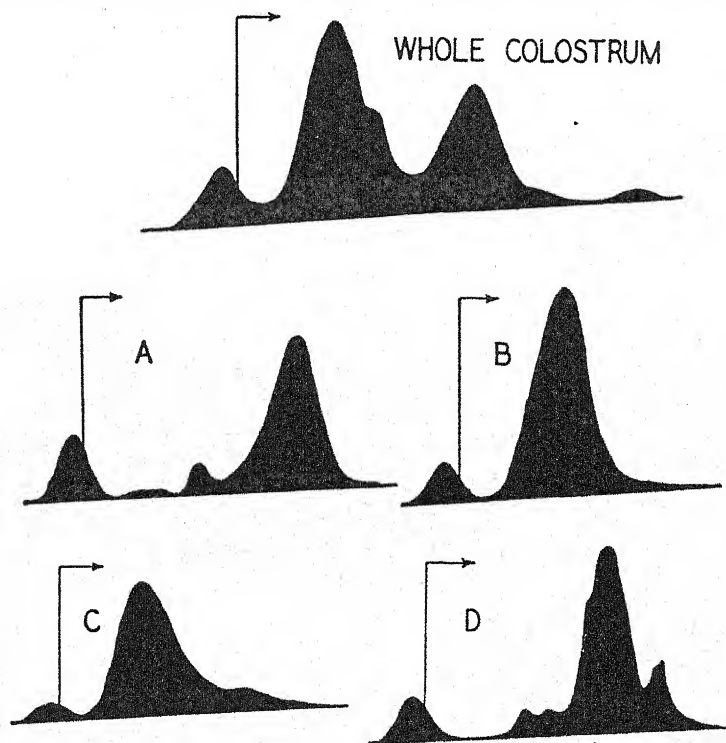


FIG. 1. Electrophoretic patterns of the descending boundaries of whole colostrum and of fractions derived from it. *A* is the casein; *B*, *C* and *D* are ammonium sulfate fractions obtained between 0 and 0.3 saturation (*B*), between 0.3 and 0.5 (*C*), and between 0.5 and 0.9 (*D*), respectively. Fraction *B* consists entirely of immune globulin and *C* mainly of this protein. The principal component of *D* is  $\beta$ -lactoglobulin, which could be obtained in crystalline form. Electrophoresis was for 200 minutes in veronal buffer at pH 8.3 to 8.4 at an ionic strength of 0.1. (Figure reproduced by permission of the Journal of Biological Chemistry.)

portion may increase somewhat in animals that have been hyperimmunized (26). The changes in protein composition during the transition from colostrum to milk have been studied by Crowther and Raistrick (5). More recently these changes also have been observed in the Tiselius apparatus (7, 14).

Because of the low concentration of immune protein in the milk whey,



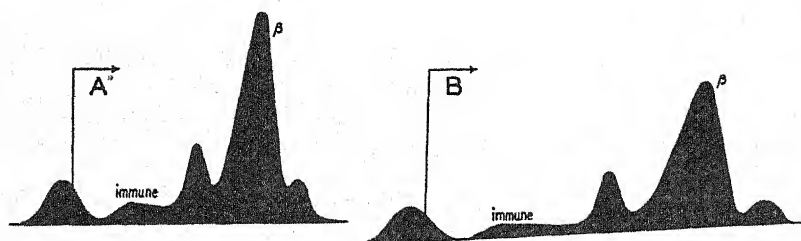


FIG. 2. Electrophoretic patterns of normal whey taken at 166 minutes (*A*) and 250 minutes (*B*). The principal component is  $\beta$ -lactoglobulin. The immune globulin represents about 10 per cent of the total protein. (Figure reproduced by permission of the Journal of Biological Chemistry.)

a somewhat different procedure from that used for the colostrum was adopted in order to isolate the immune lactoglobulin. This was accomplished

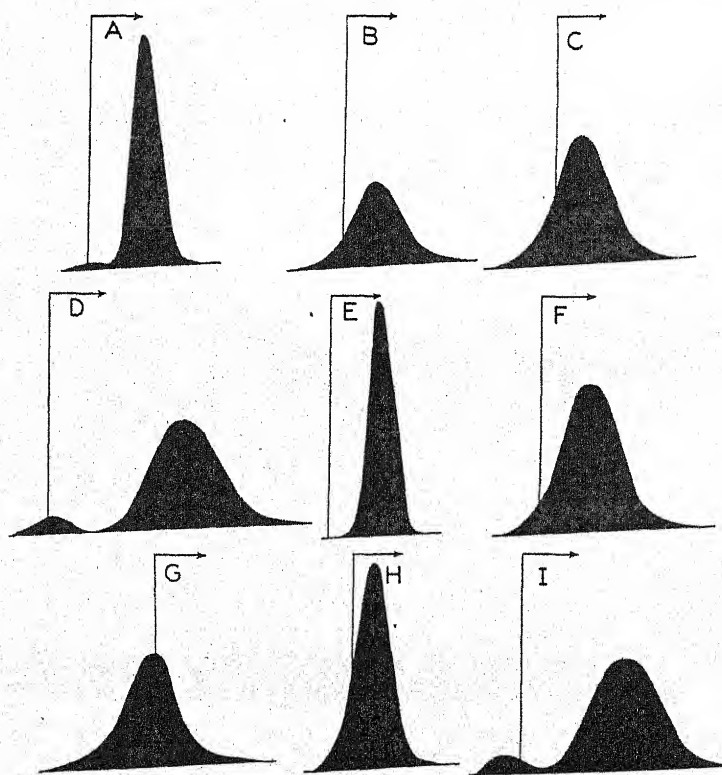


FIG. 3. Descending patterns of the purified immune lactoglobulins. The pseudoglobulin is shown in *A* (pH 3.90), *B* (pH 5.11), *C* (pH 6.81), and *D* (pH 8.55). The euglobulin is in *E* (pH 3.81), *F* (pH 5.12), *G* (pH 6.13), *H* (pH 6.82), and *I* (pH 8.65). These boundaries do not show the presence of the other milk proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

by ammonium sulfate fractionation involving isoelectric precipitations at different pH values (26). The immune lactoglobulins of milk and colostrum, as far as the authors have been able to determine, are extremely similar or, more probably, identical. Figure 3 shows the electrophoretic patterns obtained at various pH values with some of the purified immune lactoglobulins. These proteins are free from the other milk proteins. How-

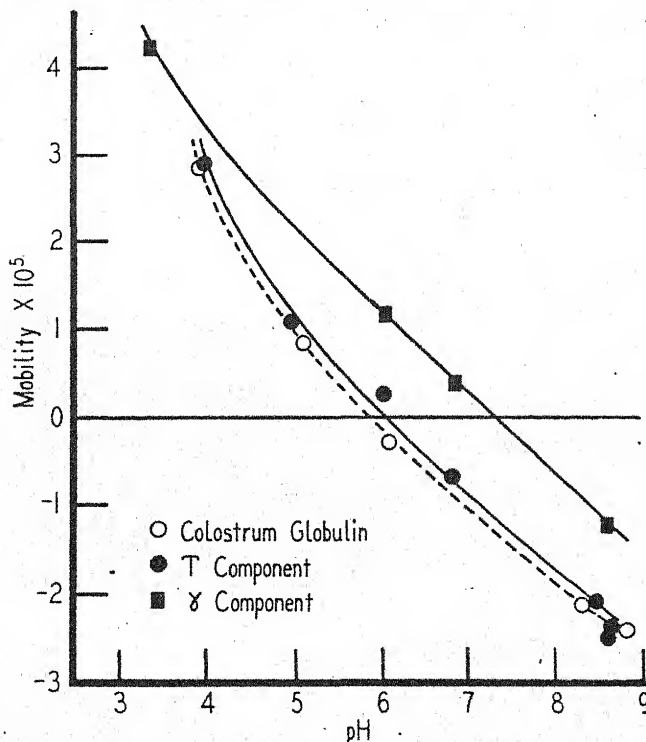


FIG. 4. Electrophoretic mobility as a function of pH for colostrum globulin, *T*-globulin, and  $\gamma$ -globulin. All of the values were calculated from descending migrations in univalent buffers at 1° C. The mobility is in sq. cm. per volt per second. The isoelectric point of the  $\gamma$ -globulin is at pH 7.2, that of the *T*-component at pH 6.15, and that of the total immune globulin of colostrum at pH 5.85. The separated lactoglobulins (not shown in the figure) gave for the pseudoglobulin an isoelectric point of pH 5.6, and for the euglobulin an I.E.P. of pH 6.05. (Figure reproduced by permission of the Journal of Biological Chemistry.)

ever, most of the boundaries show greater spreading than would be expected for a single molecular species. This is similar to the many observations that have been made with the  $\gamma$ -globulins of serum.

The animals from which samples of milk and colostrum were obtained had been hyperimmunized. All of the immune activity is associated with the specific lactoglobulins which were isolated and not with any of the other proteins of milk or colostrum, such as casein or  $\beta$ -lactoglobulin.

Since the immune proteins of bovine serum had not been previously isolated, it was necessary to develop a procedure for this purpose. As in the plasma of the horse (28, 34), immune activity is associated with the more rapidly migrating *T*-fraction as well as with the  $\gamma$ -globulin (25). The procedures developed by Cohn *et al.* (4) and Oncley *et al.* (16) for the fractionation of normal human plasma were adapted for the fractionation of hyperimmune bovine serum. This permitted the isolation of the  $\gamma$ - and *T*-globulins in electrophoretically homogeneous form (25). With these proteins in hand, it then was possible to compare the bovine immune proteins obtained from the different body fluids.

Figure 4 shows the electrophoretic mobilities as a function of pH for the bovine immune proteins. The  $\gamma$ -globulin possesses a much higher isoelectric point than the *T*-globulin or the total colostrum immune lactoglobulin. The eu- and pseudoglobulins of the colostrum or milk have slightly different isoelectric points, but these fall on either side of those of the *T*-globulin. From these facts alone it is possible to say that the immune proteins found in milk and colostrum are not  $\gamma$ -globulins as defined in terms of electrophoretic mobility and isoelectric point. However, it is not possible from these measurements to differentiate the lactoglobulins and the *T*-globulins.

While none of these proteins is completely homogeneous in the ultracentrifuge, they contain roughly 80-90 per cent of a component which sediments at 7 Svedberg units. The diffusion constants of these preparations range from about 3.3 to  $3.9 \times 10^{-7}$  sq. cm. per second. From these values it may be calculated that the principal components of the bovine immune lactoglobulins and serum globulins possess molecular weights in the neighborhood of 180,000. The ready diffusibility of the immune lactoglobulins of the colostrum through the intestine of the newborn animal cannot be due to any difference in size of these proteins as compared to the immune globulins of the serum.

Some studies have been made of the carbohydrate and amino acid content of the different bovine immune globulins (29, 30). All of these proteins were found to contain hexose and hexosamine in a ratio of about 2 to 1. These proteins, as shown in table 1, contain all of the amino acids known to be required in mammalian nutrition. In general, the data are similar to the analyses of human  $\gamma$ -globulin reported by Brand *et al.* (1). The immune globulins from horse and human serum also have been analyzed (29, 30); these greatly resemble the bovine proteins in their amino acid composition, although there are enough differences to indicate the different species from which the proteins are obtained.

It is rather striking that the immune proteins of different species appear to form a definite homologous group, with extremely similar physical and



chemical properties, in much the same way as do the serum albumins and the hemoglobins. It also is noteworthy that the immune globulins are quite different in amino acid content from any of the other proteins which are known to be present in mammalian milk or serum.

Although the bovine immune proteins greatly resemble one another, it is possible to distinguish between them. For example, attention may be called to the values for arginine and methionine of  $\gamma$ -globulin, which are much higher than those for the *T*-globulin or the lactoglobulins. The leucine content of the different proteins also shows marked differences. The phenylalanine content of the *T*-globulin is higher than that of the  $\gamma$ -globulin

TABLE I

*Amino acid and carbohydrate content of bovine immune proteins*

(Data are averages derived from (25), (26), (29) and (30). All values are in terms of the anhydrous ash-free proteins.)

Constituent	Eu-lacto- globulin	Pseudo- lactoglobulin	<i>T</i> -globulin	$\gamma$ -globulin
	(%)	(%)	(%)	(%)
Arginine .....	4.9	3.5	4.8	5.8
Histidine .....	1.89	2.14	2.01	2.05
Lysine .....	6.3	7.2	6.4	6.7
Isoleucine .....	3.1	3.1	3.0	3.2
Leucine .....	10.4	9.1	8.6	7.4
Valine .....	10.4	9.4	9.5	10.0
Threonine .....	10.5	10.1	9.5	10.0
Phenylalanine .....	3.6	3.8	4.5	3.2
Tryptophane .....	2.4	2.7	2.6	2.6
Cystine .....	3.2	3.0	2.8	2.9
Methionine .....	0.98	1.08	1.00	1.18
Sulfur .....	1.05	1.04	0.95	1.02
Hexose .....	2.9	2.8	2.5	2.05
Hexosamine .....	1.45	1.32	1.50	1.31

or of the immune lactoglobulins; this is strikingly reflected in the ultraviolet absorption spectra of these proteins (27). As shown in figure 5, bovine *T*-globulin possesses a much steeper end-absorption than the colostrum or  $\gamma$ -globulin. It thus appears that the lactoglobulins possess somewhat different protein moieties than the serum immune proteins.

However, the lactoglobulins and the serum globulins must be substances very closely related. All of these proteins will give quantitatively equivalent anaphylactic cross-reactions in guinea pigs sensitized to bovine immune proteins whether they are derived from milk or from serum (25). It is well known, particularly from the works of Wells and Osborne (36), that the globulin fractions of milk and serum are immunologically related. It now has been shown that these cross-reactions are due to the immune proteins.

The presence in the immune proteins of all the amino acids known to be required for the maintenance of nitrogen equilibrium in mammals raises

an important nutritional question. Obviously, for protection of the adult and the newborn against infectious disease, all of the so-called essential amino acids must be supplied in adequate amount. Evidence is available which shows that hypoproteinemia causes a decrease of antibodies and a lower resistance to infection. The synthesis of the globulins concerned with immunity is then a special problem in nutrition, as recently emphasized by Cannon (3).

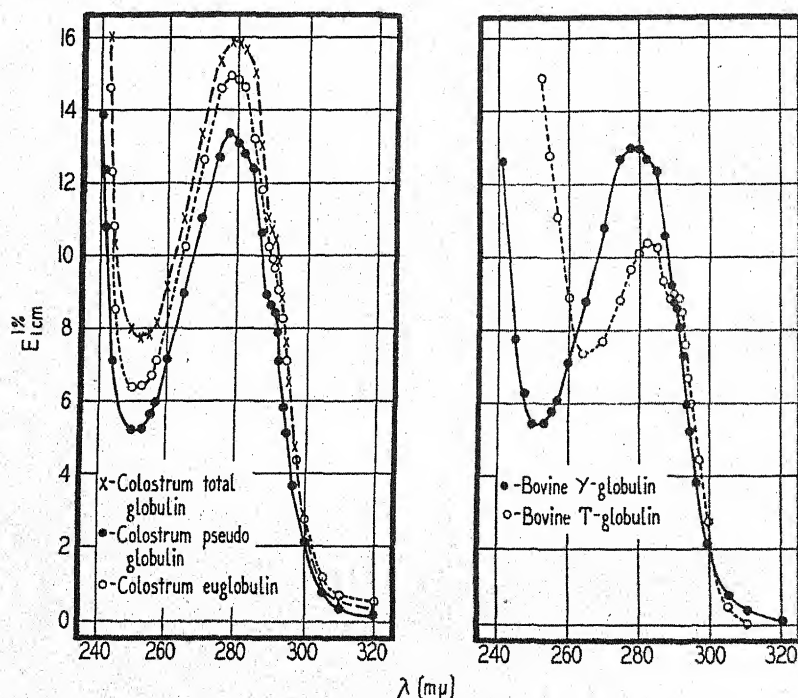


FIG. 5. Ultraviolet absorption spectra of bovine globulins. The absorption curve of the T-globulin appears to reflect the higher content of phenylalanine by the steeper end-absorption as compared to the other proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

#### PASSIVE IMMUNITY IN THE CALF

Since it already has been demonstrated that the colostrum immune globulin is different from  $\gamma$ -globulin in electrophoretic mobility and other properties, it is preferable not to refer to the globulin which appears in the blood stream of the newborn after the ingestion of colostrum as a  $\gamma$ -globulin. The protein which appears in the blood stream of the calf after ingestion of colostrum possesses the electrophoretic mobility of the immune lactoglobulin and not that of  $\gamma$ -globulin (31). Figure 6 shows the electrophoretic patterns obtained with the serum of the newborn calf at birth and

at various times later. The calf was fed colostrum only during the first day of life, and thereafter was isolated from the mother. The serum of the newborn calf did not contain any slow-moving globulin. However, the pattern of the serum obtained 2 days after birth showed a large amount of colostrum globulin (44 per cent of the total protein). Thereafter, the

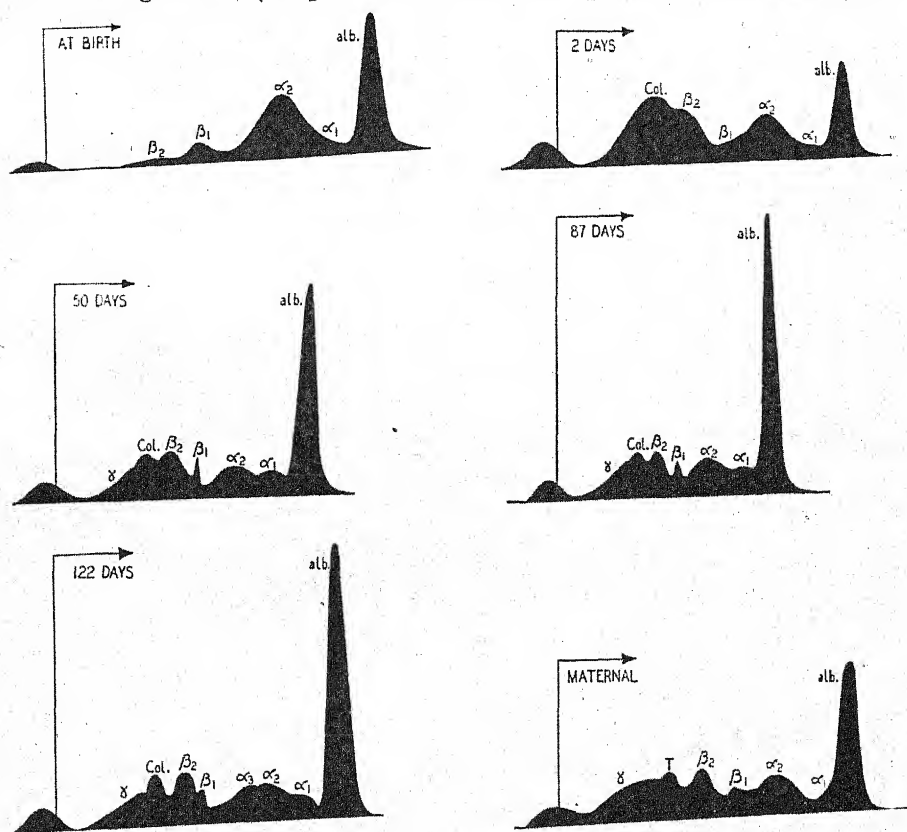


FIG. 6. Electrophoretic patterns of the descending boundaries of the serum of a newborn calf and of the same animal 2, 50, 87 and 122 days later. For comparison, the maternal serum obtained 3 days before term also is shown. The runs were performed at 1° C. in a veronal buffer of pH 8.4 to 8.6, and at an ionic strength of 0.1. The serum of the newborn is practically devoid of slow-moving globulins. The immune component (Col.) appears after feeding colostrum. The absolute heights of the different serum samples cannot be compared as the runs were made at somewhat different protein concentrations.

amount of colostrum globulin decreased steadily. From such data it is possible to estimate the time during which this protein remained in the blood stream of the calf. The immune component decreased to about one-half its initial concentration in about 20 days, and persisted for many



months.  $\gamma$ -globulin was hardly detectable in the blood stream of the newborn calf. Even after 4 months, it had not reached the normal adult level. It is obvious that the passively acquired immunity is of real importance to the health of the calf for the long period before it is capable of making antibodies of its own.

It also should be stated that the mobilities and relative concentrations of the various serum proteins in the newborn calf may be very different from those in the adult. This should be extremely useful in approaching some of the problems of the physiology and chemistry of the fetus. In fact, Pedersen (20) recently has reported the isolation of fetuin, an  $\alpha$ -globulin, from the serum of the bovine fetus. Bovine fetal hemoglobin reportedly differs from that of the adult (37).

#### SUMMARY

Colostrum serves a special function in order to enhance the resistance of the newborn to infectious disease. This is shown by the extremely high concentrations of immune lactoglobulins in the colostrum. These globulins are passively transferred to the offspring, where they may persist in the blood stream for many months. The lactoglobulins which have molecular weights near 180,000 pass from the intestinal tract of the calf to its blood stream. The immune lactoglobulins of bovine milk and colostrum, and the  $\gamma$ - and *T*-globulins of bovine serum, have been isolated and compared with respect to their physical and chemical properties.

#### CONCLUSIONS

It is clear that the colostrum serves a special function in order to enhance the survival of the newborn animal. Not only is the colostrum richer than the milk in some of the vitamins, as demonstrated by various investigators (6, 19, 23), but it also contains a totally different distribution of proteins than the milk. The extremely high percentage of immune lactoglobulins, together with the fact that the colostrum may contain 25 per cent protein in the aqueous phase, demonstrates the extreme nature of this adaptation. It is not surprising that many investigators have found that colostrum generally possesses higher immune titers than the maternal blood (2). The long duration of the passively acquired immunity in the calf also emphasizes the great importance of colostrum to the health and well-being of the newborn.

The protein synthesis of the mammary gland presents an intriguing picture. The gland makes special proteins such as casein and  $\beta$ -lactoglobulin, which are not found elsewhere in the body, and even gives its own characteristic label to the immune bodies which must be drawn from the blood stream.

## REFERENCES

- (1) BRAND, E., KASSELL, B., AND SAIDEL, L. J. Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. III. Amino Acid Composition of Plasma Proteins. *J. Clin. Invest.*, 33: 437-444. 1944.
- (2) BRAUN, H., HOFMEIER, K., AND HOLZHAUSEN, G. v. Die Vererbungsfrage in Der Lehre von Immunität Gegen Infektionskrankheiten. In Koele, W., and Wassermann, A. v., eds., *Handbuch der Pathogenen Mikroorganismen*. Aufl. 3, Bd. 1: 1109-1146. 1929.
- (3) CANNON, P. R. The Relationship of Protein Metabolism to Antibody Production and Resistance to Infection. In Anson, M. L., and Edsall, J. T., eds., *Advances in Protein Chem.*, 2: 135-154. 1945.
- (4) COHN, E. J., STRONG, L. E., HUGHES, W. L., JR., MULFORD, D. J., ASHWORTH, J. N., MELIN, M., AND TAYLOR, H. L. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. *J. Am. Chem. Soc.*, 68: 459-475. 1946.
- (5) CROWTHER, C., AND RAISTRICK, H. A Comparative Study of the Proteins of the Colostrum and Milk of the Cow and Their Relations to Serum Proteins. *Biochem. J.*, 10: 434-452. 1916.
- (6) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. II. The Carotene and Vitamin A Content of Cows' Colostrum. *Biochem. J.*, 27: 1998-2005. 1933.
- (7) DEUTSCH, H. F. A Study of Whey Proteins from the Milk of Various Animals. *J. Biol. Chem.*, 169: 437-448. 1947.
- (8) EHRLICH, P. Ueber Immunität Durch Vererbung und Säugung. *Z. Hyg. Infektionskrankh.*, 12: 183-203. 1892.
- (9) FAMULENER, L. W. On the Transmission of Immunity from Mother to Offspring. A Study upon Serum Hemolysins in Goats. *J. Infectious Diseases*, 10: 332-368. 1912.
- (10) HOWE, P. E. An Effect of the Ingestion of Colostrum upon the Composition of the Blood of New-Born Calves. *J. Biol. Chem.*, 49: 115-118. 1921.
- (11) JAMESON, E., ALVAREZ-TOSTADO, C., AND SORTOR, H. H. Electrophoretic Studies on New-Born Calf Serum. *Proc. Soc. Exptl. Biol. Med.*, 51: 163-165. 1942.
- (12) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.
- (13) MASON, J. W., DALLING, T., AND GORDON, W. S. Transmission of Maternal Immunity. *J. Path. Bact.*, 33: 783-797. 1930.
- (14) McMEEKIN, T. L., DELLAMONICA, E., AND CUSTER, J. H. Separation and Properties of Bovine Whey Proteins. *Federation Proc.*, 6: 277. 1947.
- (15) MELLANDER, O. Elektrophoretische Untersuchungen von Casein. *Biochem. Z.*, 300: 240-245. 1939.
- (16) ONCLEY, J. L., MELIN, N., RICHERT, D. A., CAMERON, J. W., AND GROSS, P. M. *J. Am. Chem. Soc.* In press.
- (17) ORCUTT, M. L., AND HOWE, P. E. The Relation between the Accumulation of Globulins and the Appearance of Agglutinins in the Blood of New-Born Calves. *J. Exptl. Med.*, 36: 291-308. 1922.
- (18) PALMER, A. H. The Preparation of a Crystalline Globulin from the Albumin Fraction of Cow's Milk. *J. Biol. Chem.*, 104: 359-372. 1934.
- (19) PEARSON, P. B., AND DARNELL, A. L. The Thiamine, Riboflavin, Nicotinic Acid and Pantothenic Acid Content of Colostrum and Milk of the Cow and Ewe. *J. Nutrition*, 31: 51-57. 1946.

- (20) PEDERSEN, K. O. Ultracentrifugal Studies on Serum and Serum Fractions. Almqvist and Wiksells Boktryckeri, Upsala, Sweden. 1945.
- (21) SAN CLEMENTE, C. L., AND HUDDLESON, I. F. Electrophoretic Studies of the Proteins of Bovine Serums with Respect to Brucella. Michigan Agr. Expt. Sta. Bull. 182. Pp. 3-44. 1943.
- (22) SCHNEIDER, L., AND SZATHMÁRY, J. I. Ueber die Immunität der neugeborenen Säugetiere. Z. Immunitätsforsch., 94: 458-464. 1938.
- (23) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat-Soluble Vitamins. XLI. The Carotene and Vitamin A Content of Colostrum. J. Biol. Chem., 107: 697-703. 1934.
- (24) SMITH, E. L. The Immune Proteins of the Cow. Federation Proc., 5: 154. 1946.
- (25) SMITH, E. L. The Immune Proteins of Bovine Colostrum and Plasma. J. Biol. Chem., 164: 345-358. 1946.
- (26) SMITH, E. L. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. J. Biol. Chem., 165: 665-676. 1946.
- (27) SMITH, E. L., AND COY, N. H. The Absorption Spectra of Immune Proteins. J. Biol. Chem., 164: 367-370. 1946.
- (28) SMITH, E. L., AND GERLOUGH, T. D. The Isolation and Properties of the Proteins Associated with Tetanus Antitoxic Activity in Equine Plasma. J. Biol. Chem., 167: 679-687. 1947.
- (29) SMITH, E. L., AND GREENE, R. D. Further Studies on the Amino Acid Composition of Immune Proteins. J. Biol. Chem., 171: 355-362. 1947.
- (30) SMITH, E. L., GREENE, R. D., AND BARTNER, E. Amino Acid and Carbohydrate Analyses of Some Immune Proteins. J. Biol. Chem., 164: 359-366. 1946.
- (31) SMITH, E. L., AND HOLM, A. The Transfer of Immunity to the New-Born Calf from Colostrum. Unpublished.
- (32) SMITH, T., AND LITTLE, R. B. The Significance of Colostrum to the New-Born Calf. J. Exptl. Med., 36: 181-198. 1922.
- (33) SMITH, T., AND LITTLE, R. B. The Absorption of Specific Agglutinins in Homologous Serum Fed to Calves during the Early Hours of Life. Jour. Exptl. Med., 36: 453-468. 1922.
- (34) VAN DER SCHEER, J., WYCKOFF, R. W. G., AND CLARK, F. H. The Electrophoretic Analysis of Several Hyperimmune Horse Sera. J. Immunol., 39: 65-71. 1940.
- (35) WARNER, R. C. Separation of  $\alpha$ - and  $\beta$ -Casein. J. Am. Chem. Soc., 66: 1725-1731. 1944.
- (36) WELLS, H. G., AND OSBORNE, T. B. Anaphylaxis Reactions with Purified Proteins from Milk. J. Infectious Diseases, 29: 200-216. 1921.
- (37) WYMAN, J., JR., RAFFERTY, J. A., AND INGALLS, E. N. Solubility of Adult and Fetal Carbohemoglobin of the Cow. J. Biol. Chem., 153: 275-284. 1944.



## THE UTILIZATION OF WHEY: A REVIEW

BYRON H. WEBB AND EARLE O. WHITTIER

*Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural  
Research Administration, U. S. Department of Agriculture*

### CONTENTS

The whey disposal problem .....	140
Quantities of whey produced .....	141
Composition and nutritive value of whey .....	141
Processes for primary products .....	142
Pasteurized products .....	144
Sweet whey .....	144
Whey cream .....	144
Concentrated products .....	144
Plain condensed whey .....	144
Sweetened condensed whey .....	145
Dried whey .....	145
Lactose .....	147
Whey protein .....	150
Fermentation products .....	151
Lactic acid .....	151
Ethyl alcohol .....	152
Riboflavin .....	152
Utilization of primary products .....	153
Feed uses .....	153
Fluid, concentrated and dried whey .....	153
Food uses .....	153
Foams and emulsifiers .....	154
Whey drinks .....	154
Soups .....	155
Cheese and cheese foods .....	155
Bakery products .....	156
Candies .....	157
Spirit vinegar .....	157
Food acidulant .....	157
Whey butter .....	157
Pharmaceutical uses .....	158
Lactose .....	158
Riboflavin concentrates .....	158
Penicillin .....	158
Hydrolyzed lactose .....	159
Hydrolyzed protein .....	159
Therapeutic products .....	160
Chemical uses .....	160
Lactic acid .....	160
Butyl alcohol .....	161

The purpose of this review is to make available in one article pertinent information on whey and its constituents and on procedures for the manu-

facture of both food and non-food products from whey. Sufficient details are given so that cheese producers can select processes suited to their individual locations and scales of operation.

#### THE WHEY DISPOSAL PROBLEM

More than ever before producers of cheese are seeking new methods of utilizing whey. The reasons for this are apparent. Older methods of disposal are becoming less available and several other possible methods are prohibitively expensive to operate. The following procedures either have been employed or have been advocated for disposal of dairy wastes (124):

(a) Running to the sewer. This is practical only when the quantity of whey is very small or the whey can be diluted greatly; otherwise, a nuisance is created or, if the sewage is treated, an inordinate load is placed on the treating plant. Since the biological oxygen demand of whey is high and the quantity usually is large, this method is not feasible in most instances.

(b) Running to a stream. Any appreciable quantity of whey will pollute a small stream sufficiently to kill fish and to produce noxious odors in stagnant areas.

(c) Dumping in abandoned mines or quarries or in holes dug for disposal purposes. The cost of transporting whey to abandoned mines or quarries will be prohibitive in most instances and the odors produced by the decomposing whey will be objectionable unless the place of disposal is at a considerable distance from the cheese plant and from homes.

(d) Dumping in prepared lagoons. Practically the same objections apply to lagooning or spreading on fields as to the preceding method.

(e) Treating in a sewage disposal plant installed for the purpose. The cost of a disposal plant for whey will be unreasonably great because of the high value of the biological oxygen demand of the whey.

(f) Producing fuel gas by anaerobic fermentation. It appears theoretically possible to produce all the heat required in a cheese plant by this procedure, but such a heat source probably would be unreliable. Digestion tanks of about 30 times the daily volume of whey would be required.

(g) Returning the whey to farmers for feeding. This is practical, but only to a limited extent. The large volume of the whey in relation to the quantity of nutrients present and the requirement that whey may not be transported to the farms in the cans used for bringing milk to the plants discourage many farmers from using whey for feeding. Furthermore, the farmers having pigs to feed usually are not the same ones as those delivering milk to the cheese plant.

(h) Evaporating or drying for use as food or feed. Where cheap heat is available and the volume of whey is great enough to justify investment in evaporating or drying equipment, this is a practical means of disposal.

(i) Manufacturing of such products as whey protein, whey cheese, lac-

tose, lactic acid, alcohol, vinegar and food specialties. This and the preceding method are the most desirable from the standpoint of nutritional economy and usually can be operated at the least net cost, and frequently at a profit. The special equipment required is expensive.

#### QUANTITIES OF WHEY PRODUCED

Approximately 10 billion lb. of whey are produced each year in the United States. About 9 billion lb. are from the manufacture of whole milk cheese and one billion lb. from cottage, pot and bakers' cheese. The 40 million-lb. quantity of whey from the making of casein, though relatively small in amount, is of importance because, until recently, it has been the source of all the lactose produced in this country.

Ten billion pounds of whey contain, in round numbers, 500 million lb. of lactose, 50 million lb. of protein, 40 million lb. of non-protein nitrogenous matter, 30 million lb. of fat, 11 million lb. of phosphorus ( $P_2O_5$ ), 7 million lb. of calcium ( $CaO$ ), and 12 thousand lb. of riboflavin. Forty million pounds of ash constituents also are present but are of little or no interest from the standpoint of utilization. The fat can be recovered readily by means of a cream separator for use in making butter and therefore does not contribute to the whey disposal problem.

#### COMPOSITION AND NUTRITIVE VALUE OF WHEY

A typical cheese whey contains 6.9 per cent total solids, of which percentage 0.6 is ash and 6.3 is organic solids, divided among 0.3 per cent fat, 0.9 per cent nitrogenous compounds (calculated as protein), 4.9 per cent lactose and 0.2 per cent lactic acid. The lactic acid has been formed by fermentation of lactose, and the percentages of these two constituents are somewhat variable, but their sum is consistently close to 5.1 per cent. About five-ninths of the nitrogenous matter is heat-coagulable protein. This protein commonly is called either whey protein or albumin, but the term albumin is an improper one since this material consists of a very small proportion of suspended casein, an "albumin fraction" and a "globulin fraction". These fractions differ in both composition and physical properties and can be fractionated still further.

For the purposes of this review, the term whey protein will include all of the heat-coagulable protein of whey, and its heterogenous composition will be disregarded. The nitrogenous matter that is not coagulable by heat consists of substances precipitable by trichloroacetic acid, which are peptone or proteose in nature, and other simpler substances such as creatin, creatinin, urea, uric acid, amino acids and ammonia. Of the known vitamins, the only one present in appreciable quantities in whey is riboflavin, which occurs to the extent of approximately 1.24  $\gamma$  per g. of whey, or 0.000124 per cent (25).

The chief individual ash constituents present in whey are: 0.188 per cent



potassium oxide, 0.075 per cent sodium oxide, 0.071 per cent calcium oxide, 0.018 per cent magnesium oxide, 0.001 per cent ferric oxide, 0.110 per cent phosphorus pentoxide, 0.107 per cent chlorine and 0.029 per cent sulfur trioxide. Of these, the calcium and phosphorus are of positive interest because of their nutritional value. The other salt constituents usually have only nuisance value because of the salty flavor that they impart to concentrated whey products and the difficulty of removing them.

Milk is unique as a source of calcium and riboflavin, two nutrients that need to be increased in many American diets. It is unique also in that it contains lactose, a sugar having highly specific nutritive value. These three nutrients largely are left in the whey from the cheesemaking process, together with part of the phosphorus. The whey protein is of excellent quality in that it contains practically all of the essential amino acids.

Lactose brings about increased utilization of calcium, magnesium and phosphorus in young animals (35, 72, 81). This effect may be the real basis for many claims as to the superior assimilability of calcium and phosphorus from whey products. Lactose, unless fed in excessive quantities, is more effective in accelerating growth in young animals than are other common carbohydrates (141). It favors the production of riboflavin and vitamin B<sub>6</sub> in the intestine of the rat (74). The feeding of lactose to rats has caused cataracts, but it has been found that fat, which is necessary for the utilization of dietary lactose, protects against development of cataracts (51, 103). Young rats die when fed lactose in high concentration as the only carbohydrate in the diet (31, 40) unless an unidentified factor associated with casein is present (18). It should be understood that these undesirable effects of lactose have been obtained only in rats and then only on diets that were highly abnormal.

In the poultry industry, dried whey is fed extensively because lactose is effective in preventing coccidiosis, and riboflavin is considered essential to the rapid growth of chicks and to hatchability of eggs and is a preventive of curled toe paralysis.

#### PROCESSES FOR PRIMARY PRODUCTS

Methods of whey utilization discussed in this review are shown in figure 1. Three primary processes are employed in the preparation of whey for ultimate utilization in feeds, foods, pharmaceuticals, or industrial products. The different primary and final products will be considered here approximately in the order in which they appear in the figure.

If whey is to be processed, the initial processing should be carried out in the plant in which the whey is produced in order that deterioration due to the growth of undesirable organisms will be retarded or prevented. Such treatments may include one or more of the unit operations of pasteurization, concentration or fermentation.

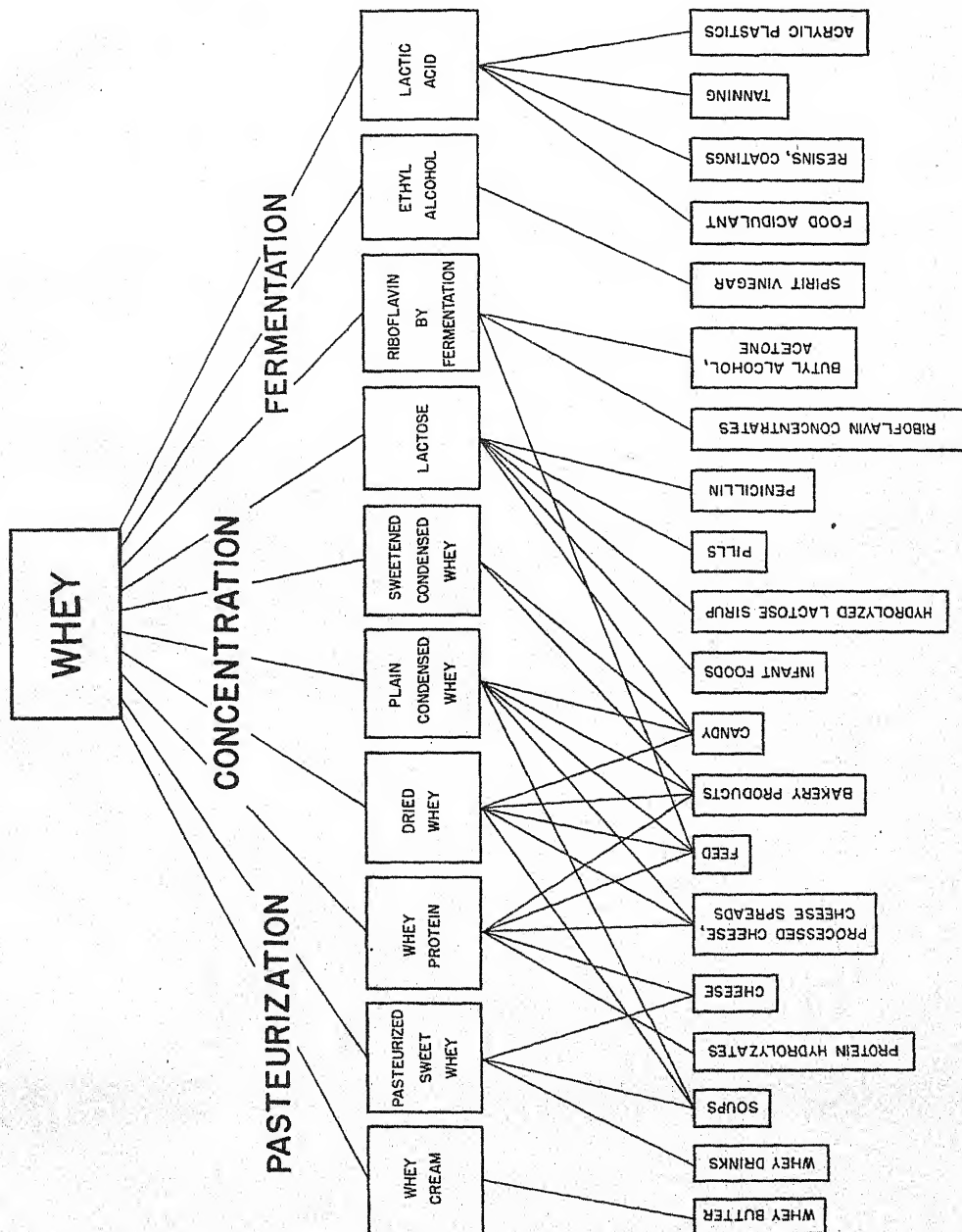


Fig. 1. Flow sheet of products from whey.

### *Pasteurized Products*

*Sweet whey.* Pasteurization is an essential step in the preservation of sweet whey for further processing and in the production of whey cream. Whey is heated to 145° F. for 30 minutes, or to higher temperatures for shorter times, to retard growth of lactic-acid-producing bacteria which have been active in the cheesemaking process and to destroy pathogenic organisms that may be present if the milk was not pasteurized prior to making the cheese. The whey should be cooled to below 50° F. immediately after pasteurization, unless additional processing is to ensue promptly.

Pasteurized sweet whey is bulky and perishable, but, where there is a convenient source of supply, it can be used successfully in foods. It may be used to advantage in place of water in recipes for beverages, soups, bread and other foods.

*Whey cream.* It is prepared by putting whey through a cream separator either before or after pasteurization. Products of bacterial growth that produce off flavors may be present in whey that is not properly pasteurized and may be transferred to the cream. When cheese is made in copper equipment, cream made from the whey often has a copper content great enough to catalyze the oxidation of the fat; as a result, off flavors appear in butter or other products made from the cream.

The danger of curdling during the pasteurization of mixtures of whey cream and cream from whole milk can be lessened or avoided by using whey cream of low acidity or by pasteurizing the whey cream separately before it is mixed with the cream from whole milk (123).

Although butter most generally is the product made from whey cream, the cream may be used in foods in which casein coagulates readily. Whey cream contains substantially no casein and will not produce a coagulum when added to cold, acid foods. The coagulum formed during heating is soft and easily dispersed.

### *Concentrated Products*

To remove water from whey, two methods are in use, one employing vacuum evaporators and the other driers. Both require from 40,000 to 100,000 lb. of whey daily for profitable operation. However, if equipment already is available and if the whey cannot be discarded, it may be practicable to concentrate as little as 10,000 lb. of whey daily. The engineering and operating aspects of concentrating equipment have been considered at length by Hunziker (46), Scott (104) and Farrall (32).

*Plain condensed whey.* This is the simplest of the whey concentrates to manufacture (136). Pasteurized whey is condensed in a vacuum pan to about 68 per cent solids (36.7° Baumé at 115° F.), dropped into cans, barrels or a tank, seeded with lactose or a concentrate from a previous run, and, if possible, cooled with agitation. A multiple-effect evaporator will



condense whey to 40 or 50 per cent solids; a single-effect pan can reduce it to 70 per cent solids. When whey is being condensed to 70 per cent solids, there is a possibility that the concentrated solution suddenly will crystallize in the pan and that the heating surfaces will become badly coated with whey solids (60). To avoid this difficulty, the pan should be clean and free of lactose crystals at the start of each run and the batch should be quickly finished and dropped from the pan (136).

Excessive foaming of whey in the vacuum pan sometimes occurs. The addition of a small quantity of milk fat or other fat to the batch usually will reduce this foaming. Triggs (125) found that if the whey was adjusted to pH 5.5 to 6.0 before it was drawn into the vacuum pan, foaming could be practically eliminated. The acid preferred was phosphoric, and this was neutralized with lime as soon as the concentrated whey was dropped from the pan.

*Sweetened condensed whey.* This was developed to provide a simple and economical method for the preservation of whey solids for use in food manufacture (92, 137). A quantity of sugar equal in weight to that of the solids in the batch of whey is added to the whey after it has been run through the separator and pasteurized. The mixture is condensed under vacuum to 76 per cent solids (38.4° Baumé at 122° F.). The concentrate is cooled to 95° F., seeded with lactose crystals or with a concentrate from a previous run, stirred for 1 hour and placed in barrels or cans. If a product containing a reduced content of lactose is desired, crystallized lactose may be removed centrifugally before the concentrate is stored. The storage requirements, as well as the manufacturing procedure, are similar to those for sweetened condensed milk. The whey product thickens more slowly and to a lesser extent than does the milk product. Fat-free sweetened condensed whey may be whipped to a dense foam having 200 per cent overrun and a stability of several hours.

*Dried whey.* When produced by the processes employed in drying milk, dried whey cakes on standing because the anhydrous lactose present gradually absorbs water and crystallizes as a hydrate (108, 110). Many patented processes have been devised principally for the purpose of inducing lactose crystallization prior to the complete drying of the whey. Some of these processes may be applied in either the roller or spray drying procedures.

Processes for controlling the crystallization of lactose during the drying of whey may be divided into four groups: (a) Whey is concentrated to a solids content of 70 per cent or more, the lactose is allowed to crystallize, and then the mass is dried (11, 53, 60, 111). (b) The whey is dried and then allowed to absorb water to force the crystallization of the lactose (20, 30, 87, 130). (c) The whey is concentrated to the point at which it

contains approximately the quantity of water required for hydration of the lactose (6, 58, 59, 85, 88). No water is added and under carefully controlled conditions it is not necessary to remove water after the crystals have formed. In practice, however, often about 2 to 5 per cent of water must be removed after crystallization of lactose is complete. (d) A dried whey in which lactose is largely in the *beta* anhydride form is prepared by seeding a partially concentrated whey with *beta* lactose at a temperature above 200° F. and holding it at this temperature while it crystallizes and dries (20, 59).

Three general drying methods are employed in the manufacture of dried whey: tunnel or shelf drying, drum or roller drying, and spray drying. Since many of the processes and their modifications employed in the drying of whey are patented, the status of the patents in this field should be investigated before manufacturing operations are started. Bosworth (12) concentrated whey under vacuum and then dried it in air at 149° F. for food and pharmaceutical uses.

The commercial method of concentration and tunnel drying of whey is based on the Simmons patent (111), which only recently has expired: Whey is condensed under vacuum to 70 per cent solids, dropped from the vacuum pan, seeded and held not more than 24 hours to allow the lactose to crystallize. The pasty mass then is dried in a tunnel drier and ground. The finished product is relatively non-hygroscopic and the whey protein is insoluble in water. In the Kraft modification of the Simmons process (53), the whey concentrate containing 70 per cent solids is dried in air by blowing it through a series of conduits and cyclone collectors. The lactose crystallizes during this drying operation. Lavett (60) concentrates whey of 40 per cent solids to 80 per cent on a double drum drier, drops this to cooling and seeding drums, and then to a hot-air drier. Bertram and Lemmerich (11) pass the concentrated whey through a mixing machine in which it is mixed with air and the lactose crystallized. The material then is dried and ground. Peebles and Manning (89) claim that, by heat-coagulating the whey proteins prior to crystallizing the lactose in the concentrate, the protein then will not interfere with crystal formation, and a stable, non-hygroscopic dried whey will be produced.

Drum or roller drying of whey can be carried out by following conventional methods (3), but modifications generally are employed to retard the formation of a sticky, hygroscopic glass on the drums. Lavett (57, 59, 60) uses two double-drum drying units placed one above the other. Whey is concentrated in a vacuum pan to 40 per cent solids and then reduced on the upper drums to 80 per cent solids. The mass is cooled and seeded on the lower drums and finally dried in a rotary drier. When a single pair of rolls is employed, adjustment of the titratable acidity of the whey to be

tween 0.30 and 0.40 per cent before drying is helpful (61). In one modification, the drying mass is stripped from the drums when it contains from 8 to 15 per cent moisture, the lactose allowed to crystallize, and the drying finished by means of hot air (58).

Drying aids may be added to the whey. Pectic acid is used in the drying of mixtures of whey and fruit juices (143). The dried whey can be scraped off the rollers easier if a finely divided dry material, such as dried whey, is sprinkled on the semi-dried film as the rolls revolve (6). Spellacy (115), Supplee (119), and Jack and Wasson (48) found that whey dried with less difficulty when it was mixed with a material which formed a sheet as it was scraped from the drums. Skim milk, buttermilk and organic, water-insoluble, non-gelatinized substances such as ground and sifted cereals were found to be suitable drying aids. Waite (128) found that cheese whey and hydrochloric acid-casein whey could be drum-dried if they were neutralized with calcium hydroxide, but acetic acid-casein whey could be dried without neutralization.

Spray drying can be accomplished by the processes used for drying milk. However, the hygroscopic nature of spray-dried whey sometimes causes it to cake in the collection system of the drier and obstruct the passages. This tendency is lessened by inducing lactose crystallization during drying by an adaptation of one of the procedures previously described.

The addition of pectic acid to whey (143) and heat-coagulation of the whey protein (89) have been recommended as preliminary treatments for whey that is to be spray-dried.

Casein sometimes is removed from skim milk by treatment with one of several gums (2). The resulting whey is highly viscous; it may be treated with an enzyme that will act on the gum, thus reducing the viscosity of the whey and making it easier to handle in drying equipment (23).

Dried whey often gradually becomes brown subsequent to drying. Doob *et al.* (28) found this objectionable change to be associated with a high content of osmotically held moisture, high titratable acidity and low lactose content.

*Lactose.* Until recently, lactose was made in this country only from muriatic casein whey. However, the increased demands for lactose in the manufacture of penicillin during World War II, together with the simultaneous decrease in the quantity of casein whey available, made it necessary to use cheese whey as a source of lactose. The difficulties in processing cheese whey for lactose manufacture had been overcome experimentally, and several procedures were available for commercial use. The methods employing casein whey and cheese whey are outlined here.

Whey from casein precipitated by means of sulfuric acid is objectionable



because of the difficulty of removing slightly soluble metal sulfates that impart cloudiness to lactose solutions. Self-sour casein whey is not recommended because so much of the lactose has been converted to lactic acid that yields will be low. The same is true to a less degree of cottage cheese whey. In general, the less the fermentation that has taken place in the whey the greater the yield of lactose that will be obtained.

The whey protein is recovered in an insoluble condition in the process using casein whey and in those processes using cheese whey wherein the whey is clarified by boiling. This insoluble protein is suitable for feed. When a soluble protein suitable for use as food is desired, the whey should not be boiled; under these conditions the lactose obtained will contain a relatively high percentage of protein and ash, usually 1.5 per cent or more of each. Two stages of clarification and boiling are necessary to produce, with one crystallization, lactose containing less than 0.3 per cent protein and 0.3 per cent ash. The recovery of lactose usually is 3.5 to 4 lb. per 100 lb. of whey. A second crystallization is necessary in order to produce USP lactose. For many purposes, however, lactose of crude or technical grade is satisfactory and is less costly to produce.

Muriatic casein whey (78, 117) is heated to boiling in iron tanks with live steam, and lime is added during the heating until the acidity is about 0.5 per cent or the pH value is 6.2. The coagulum is allowed to settle and the clear whey is evaporated in a double-effect evaporator to a concentration of 30 per cent lactose, or 20° Baumé. The hot sirup is filtered in a filter press and is followed by the sludge from the coagulating tank. The clear sirup then is evaporated in a single-effect evaporator to about 40° Baumé, some crystallization or "graining" taking place in the evaporating pan. The hot mass is dropped to crystallizing vats, where it is agitated slowly and cooled by water circulating in a jacket. The sugar is freed from mother liquor by spinning in a sugar centrifuge and then washed with cold water. A second crop of crystals can be obtained by concentrating the mother liquor. The wet crude lactose either should be refined or dried promptly to avoid spoilage.

The simplest method of making lactose from cheese whey (9, 10, 138) is to concentrate it in a vacuum evaporator to 55-60 per cent solids content, cool the concentrate with occasional stirring in a vat, separate the lactose in a centrifuge, wash with cold water, and dry in a tunnel drier. The resulting crude sugar will contain approximately 5 per cent impurities (protein and ash) on a dry basis. The whey protein in the mother liquor will be soluble.

Better grades of lactose are obtained when the heat-coagulable fraction is removed from the whey before concentration. During evaporation of the clarified whey further precipitation of insoluble protein and salts occurs.

If the sirup is filtered when its concentration reaches 20° Baumé, or about 30 per cent solids, there will be a further improvement in the finished product. The 20° Baumé sirup also may be decolorized with activated carbon and bone-black to produce a colorless lactose. The resulting technical grade sugar will contain more than 99 per cent lactose and only 0.3 per cent each of protein and ash on the dry basis. A lactose that will not foam in solution may be made by digesting the clarified whey with the enzyme trypsin before concentration.

Several other methods that have not come into commercial use have been described in the literature (142).

For refining (118), crude sugar is dissolved with the aid of steam in sufficient water to give a concentration of 20° Baumé. One pound of decolorizing paste and 0.25 lb. of a filter aid are added for each 100 lb. of sugar present. The solution is heated to boiling and hydrochloric acid is added to give a titratable acidity of 0.09 per cent, expressed as lactic acid. After standing overnight, the batch is heated nearly to boiling and milk of lime added to reduce the acidity to 0.05 per cent, or a pH value of 5.4 to 5.8. The solution then is boiled vigorously for a few minutes and allowed to stand until the insoluble matter has settled. It then is filtered through cloth in a press and again through rag paper supported between perforated copper discs. The acidity of the filtrate is increased slightly by addition of hydrochloric acid, the solution is concentrated to 40° Baumé, and the sugar is crystallized, centrifuged, washed and dried. The product should satisfy the specifications for USP lactose.

Drying lactose solutions by the spray-drying process (8) produces a mixture of the two forms in approximately the equilibrium ratio of 1.65 parts *beta* to 1.00 part *alpha*. The product dissolves much more rapidly than *alpha* lactose but is hygroscopic and has poor wetting properties. The product made by drying lactose solutions on a drum drier will contain as much as 90 per cent of the sugar in the *beta* form under the most favorable drying conditions. Such a product is less hygroscopic than the spray-dried product, has good wetting properties, and is slightly more soluble initially than pure *beta* lactose.

In the process of Supplee and Flanigan (120), a solution of lactose is dried in a thin film at a temperature above the boiling point of water, the film is removed from the source of heat while it contains at least 2 per cent of water, and the heat remaining in the paste completes the drying. The product contains a high proportion of *beta* lactose.

In Sharp's process (106, 107), *alpha* lactose is added to a saturated lactose solution maintained above the critical temperature of 200.4° F. The *alpha* lactose dissolves and reappears as crystalline *beta* lactose, which is separated by filtration in a heated centrifuge.

In the Sharp and Hand process (109), dry *alpha* lactose is heated in a closed container at 248 to 266° F. Under these conditions, *alpha* lactose loses water of crystallization and changes to the *beta* form. When the conversion has reached completion, or a desired stage short of completion, the water vapor in the container is allowed to escape.

*Whey protein.* This easily can be recovered as a denatured protein from either concentrated or unconcentrated whey by heat coagulation, but the soluble product is difficult to isolate. Soluble whey protein generally is prepared from a whey concentrate from which the crystallized lactose has been removed. The remaining liquor contains the whey protein, some lactose, and the soluble whey salts, which are difficult to separate from the protein without causing denaturation. Patents were obtained by Dunham in 1902 (29) for precipitating whey albumin from concentrated whey by means of acid, by Weimar in 1921 (139) for preparing a soluble whey protein concentrate from which lactose was partly separated by crystallization and salts by dialysis, and by Meyer in 1931 (71) for removing salts by chemical means.

Bell and Peter (9) and Bell *et al.* (10) improved the Weimar process. Cheese whey is neutralized with sodium hydroxide to pH 7.3, condensed to 62 per cent solids, cooled, and centrifuged to separate the lactose. The mother liquor, which contains soluble whey protein, milk salts and residual lactose, is suitable for use in food preparations. Watson (131) was able to remove most of the salts from the mother liquor of the Bell, Peter and Johnson process by electrodialysis. Perhaps this can be accomplished more readily by application of the more recently developed ion-exchange procedures, first advocated by Lyman (68).

Leviton (62) and Leviton and Leighton (67) extract soluble protein from whey by means of alcohol. Dried whey containing non-crystalline lactose rapidly is mixed with ethanol, and the protein, being insoluble in the alcohol, promptly is separated by filtration and dried. The dried product is soluble in water. Lactose crystallizes from the mother liquor and is recovered by filtration; the alcohol is recovered by distillation, leaving a residue relatively rich in riboflavin. Similarly, Leviton (66) has extracted protein from dried skim milk by means of methanol. The protein complex is redispersed in water and the casein is precipitated by acid or rennet, leaving soluble whey protein in solution.

Another method for the separation of water-soluble protein from whey was devised by Gordon (38). The protein is precipitated at pH 3 by addition of a soluble metaphosphate, separated by filtration, and washed and treated with excess calcium hydroxide at pH 9 to precipitate calcium phosphate. The mixture then is adjusted to pH 7 and centrifuged. The filtrate contains the whey protein and is evaporated under vacuum to yield the protein in a dry undenatured state.



Water-insoluble whey protein may be separated from whey by heat coagulation. Investigators have determined quantitatively the effect of reaction and temperature on the heat coagulation of protein in cheese whey (47, 76, 80). In general, 50 to 60 per cent of the nitrogen in whey is recovered as coagulated protein when the whey at a reaction between pH 4.5 and 5.0 is boiled. Several practical processes have been developed (15, 34, 130). According to Burkey and Walter (16), sweet whey (pH 6.3) is heated to 200° F. and acidified to pH 5.0 with any suitable acid or with sour whey. The sour whey used should contain 2 per cent lactic acid and is added in an amount equal to 10 per cent of the whey being treated. During acidification the whey is stirred; then it is held hot and without stirring for about 15 minutes. The clear liquid may be siphoned or drained off, or the flocculated protein dipped into cheese cloths and drained and washed. Curd that has been drained and washed contains more than 74 per cent moisture and may be preserved by drying or freezing. The clarified whey remaining after removal of the protein may be used for lactose manufacture or for animal feed.

Centrifugal separation and washing of heat-coagulated whey protein have been accomplished by means of specially built, high-speed centrifuges. The composition of the protein suspension produced may vary widely. One procedure produces a concentrate containing about 2 per cent whey protein and very small quantities of lactose and salts. Some of these suspensions resemble skim milk in appearance and can be used in food manufacture either directly or after concentration by vacuum evaporation or by drying.

#### *Fermentation Products*

The substances that can be produced by the fermentation of the lactose of whey can be produced by fermentation of cane, beet or corn sugar. Whether it is practical to utilize whey in making fermentation products depends in general on whether a suitable organism is available to convert lactose into the desired product and whether whey is a less costly source of fermentable sugar than is molasses or corn sugar.

Of the many substances that can be produced by fermentation of lactose, the only ones being produced in this country are lactic acid, ethyl alcohol and riboflavin. Lactose is used in penicillin production because its slow rate of acid production under the required conditions favors increased formation of penicillin, but it is not essential to the fermentation. Since lactose, rather than whey, is used in this fermentation, the process is not described here.

*Lactic acid.* It is produced commercially from whey by means of a mixed culture of a lactobacillus and a mycoderma, American Type Culture Collection no. 9223 (17, 49, 140, 142). The efficiency of conversion is greater than 90 per cent; the acid is the inactive mixture of the dextro and levo forms, and no objectionable by-products are formed.

In the Sharp and Hand process (109), dry *alpha* lactose is heated in a closed container at 248 to 266° F. Under these conditions, *alpha* lactose loses water of crystallization and changes to the *beta* form. When the conversion has reached completion, or a desired stage short of completion, the water vapor in the container is allowed to escape.

*Whey protein.* This easily can be recovered as a denatured protein from either concentrated or unconcentrated whey by heat coagulation, but the soluble product is difficult to isolate. Soluble whey protein generally is prepared from a whey concentrate from which the crystallized lactose has been removed. The remaining liquor contains the whey protein, some lactose, and the soluble whey salts, which are difficult to separate from the protein without causing denaturation. Patents were obtained by Dunham in 1902 (29) for precipitating whey albumin from concentrated whey by means of acid, by Weimar in 1921 (139) for preparing a soluble whey protein concentrate from which lactose was partly separated by crystallization and salts by dialysis, and by Meyer in 1931 (71) for removing salts by chemical means.

Bell and Peter (9) and Bell *et al.* (10) improved the Weimar process. Cheese whey is neutralized with sodium hydroxide to pH 7.3, condensed to 62 per cent solids, cooled, and centrifuged to separate the lactose. The mother liquor, which contains soluble whey protein, milk salts and residual lactose, is suitable for use in food preparations. Watson (131) was able to remove most of the salts from the mother liquor of the Bell, Peter and Johnson process by electrodialysis. Perhaps this can be accomplished more readily by application of the more recently developed ion-exchange procedures, first advocated by Lyman (68).

Leviton (62) and Leviton and Leighton (67) extract soluble protein from whey by means of alcohol. Dried whey containing non-crystalline lactose rapidly is mixed with ethanol, and the protein, being insoluble in the alcohol, promptly is separated by filtration and dried. The dried product is soluble in water. Lactose crystallizes from the mother liquor and is recovered by filtration; the alcohol is recovered by distillation, leaving a residue relatively rich in riboflavin. Similarly, Leviton (66) has extracted protein from dried skim milk by means of methanol. The protein complex is redispersed in water and the casein is precipitated by acid or rennet, leaving soluble whey protein in solution.

Another method for the separation of water-soluble protein from whey was devised by Gordon (38). The protein is precipitated at pH 3 by addition of a soluble metaphosphate, separated by filtration, and washed and treated with excess calcium hydroxide at pH 9 to precipitate calcium phosphate. The mixture then is adjusted to pH 7 and centrifuged. The filtrate contains the whey protein and is evaporated under vacuum to yield the protein in a dry undenatured state.

Water-insoluble whey protein may be separated from whey by heat coagulation. Investigators have determined quantitatively the effect of reaction and temperature on the heat coagulation of protein in cheese whey (47, 76, 80). In general, 50 to 60 per cent of the nitrogen in whey is recovered as coagulated protein when the whey at a reaction between pH 4.5 and 5.0 is boiled. Several practical processes have been developed (15, 34, 130). According to Burkey and Walter (16), sweet whey (pH 6.3) is heated to 200° F. and acidified to pH 5.0 with any suitable acid or with sour whey. The sour whey used should contain 2 per cent lactic acid and is added in an amount equal to 10 per cent of the whey being treated. During acidification the whey is stirred; then it is held hot and without stirring for about 15 minutes. The clear liquid may be siphoned or drained off, or the flocculated protein dipped into cheese cloths and drained and washed. Curd that has been drained and washed contains more than 74 per cent moisture and may be preserved by drying or freezing. The clarified whey remaining after removal of the protein may be used for lactose manufacture or for animal feed.

Centrifugal separation and washing of heat-coagulated whey protein have been accomplished by means of specially built, high-speed centrifuges. The composition of the protein suspension produced may vary widely. One procedure produces a concentrate containing about 2 per cent whey protein and very small quantities of lactose and salts. Some of these suspensions resemble skim milk in appearance and can be used in food manufacture either directly or after concentration by vacuum evaporation or by drying.

#### *Fermentation Products*

The substances that can be produced by the fermentation of the lactose of whey can be produced by fermentation of cane, beet or corn sugar. Whether it is practical to utilize whey in making fermentation products depends in general on whether a suitable organism is available to convert lactose into the desired product and whether whey is a less costly source of fermentable sugar than is molasses or corn sugar.

Of the many substances that can be produced by fermentation of lactose, the only ones being produced in this country are lactic acid, ethyl alcohol and riboflavin. Lactose is used in penicillin production because its slow rate of acid production under the required conditions favors increased formation of penicillin, but it is not essential to the fermentation. Since lactose, rather than whey, is used in this fermentation, the process is not described here.

*Lactic acid.* It is produced commercially from whey by means of a mixed culture of a lactobacillus and a mycoderm, American Type Culture Collection no. 9223 (17, 49, 140, 142). The efficiency of conversion is greater than 90 per cent; the acid is the inactive mixture of the dextro and levo forms, and no objectionable by-products are formed.



A starter culture is built up by successive inoculations and incubations of batches of whey of increasing size. Five hundred gallons of starter are added to 5,000 gallons of raw whey maintained at 43° C. (110° F.). Every 6 hours, or whenever the reaction approaches pH 5.0, a slurry of slaked lime is added in quantity sufficient to bring the reaction to pH 6.0 but not higher. When chemical tests show that practically all the sugar has been fermented, or when the quantity of lime consumed indicates that the conversion is complete, the whey is neutralized to pH 6.5 to 7.5 with lime slurry and heated to the boiling point. After 10 minutes at the boiling point, the coagulum is allowed to settle, the clear liquid is run to a filter press and is followed by the sludge. The hot filtrate is treated with a small percentage of decolorizing carbon, stirred, and brought to a pH value of 10.0 by addition of lime slurry. As soon as a test on a sample shows that sedimentation will be rapid, the precipitate is allowed to settle and the batch again is filtered. The filtrate is neutralized with lactic acid and concentrated in a vacuum pan at 15° Baumé. The concentrate is run to jacketed crystallizers, and, by circulating cold water in the jacket, it is cooled to 10–15° C. (50–60° F.). After 12 hours, the crystalline mass is spun in a basket centrifuge until no more filtrate is obtained and the crystals are washed lightly with cold water. The mother liquor and washings are concentrated to 13.5° Baumé to obtain a second crop of crystals. The calcium lactate obtained may be recrystallized to produce USP calcium lactate or it may be treated with sulfuric acid to convert it to lactic acid either before or after crystallization.

Lactic acid comes on the market principally as 22 and 44 per cent crude, 50 per cent edible, and 65 per cent USP acid.

*Ethyl alcohol.* The production of ethyl alcohol from the lactose of whey (50, 77, 97) requires a lactose-fermenting yeast. *Torula cremoris*, American Type Culture Collection no. 2512, is the most efficient yeast found for the purpose; it produces 84 to 90 per cent of the theoretical yield.

The whey is heated to boiling, acidified to pH 5.0 with sour whey or acid, and the precipitated protein removed by filtering. After the filtrate has cooled to 33–34° C. (93° F.), 1 lb. of the yeast is added for each 120 gallons of whey, and the fermentation continued at constant temperature until it is complete, usually for about 50 hours. The yeast is removed and the alcohol recovered by distillation. The protein, spent yeast and residues from the still are suitable for feed. The alcohol is of sufficiently good quality to be used for the production of spirit vinegar, as described later.

*Riboflavin.* Dried whey is fed to chickens not alone for its protein and lactose contents but also for its riboflavin content. During World War II when drying equipment was being used extensively in drying whole and skim milk, the difficulty of producing enough dried whey to satisfy the

needs for riboflavin in feeds was overcome by increasing the riboflavin content of whey before drying by means of a fermentation process (4, 65, 69, 73, 96, 144).

The reaction of raw whey is adjusted to between pH 6.0 and 7.0, and its iron content adjusted to between 1 and 2 parts per million. Five pounds of corn meal and 2 lb. of calcium carbonate are added for each 1,000 lb. of whey and the mixture sterilized by heating under pressure at 250° F. for 15 to 20 minutes. After the whey has been cooled to 100° F., 50 lb. of an active starter of *Clostridium acetobutylicum* (American Type Culture Collection no. 824 or other suitable strain) is added for each 1,000 lb. of whey and fermentation allowed to continue at 86 to 98° F. for 48 hours, or until riboflavin concentration no longer increases. A yield of at least 30  $\gamma$  of riboflavin per g. of whey can be expected. About 30 per cent of the lactose of the whey is converted during the fermentation to alcohols and acetone. Of these compounds, two-thirds is butyl alcohol, which is of sufficient value to warrant recovery by distillation. It usually is not feasible to recover the small quantities of ethyl alcohol and acetone present.

#### UTILIZATION OF PRIMARY PRODUCTS

##### *Feed Uses*

*Fluid, concentrated and dried whey.* The use of whey in feeding animals has been mentioned briefly at the beginning of this review. It should be emphasized that condensed and dried whey are the forms most practical to use in feeding pigs and chickens (105, 126), especially those that are being raised on farms distant from cheese factories. Dried whey is considered especially useful in feeding chickens because of the effects of lactose in preventing coccidiosis and the effects of riboflavin on growth of chicks, hatchability of eggs, and prevention of curled toe paralysis. The riboflavin content of whey that is to be dried for chicken feed can be increased advantageously prior to drying by the fermentation procedure outlined in the preceding section.

The concentrated and dried forms of whey usually are fed in mixtures with other feed materials, or the mixing may be done prior to concentration (75, 83). Sweet or soured whey, preferably somewhat concentrated, is recommended as an addition in the making of silage, especially grass silage to which it furnishes nutrients and lactic acid as a preservative (1, 45, 116).

##### *Food Uses*

An early record of the benefits to health to be gained through the consumption of whey as a food is found in Hoffmann's "Treatise on the Virtues and Uses of Whey", published in 1761 (44). But, although whey long has been offered as a cure for many illnesses, it is only recently that serious efforts have been made to incorporate it in foods.

The use of whey products in food manufacture affords a much more efficient means for utilization of whey from the standpoint of human nutrition than does feeding to animals followed by consuming the animals as food. The food value of the solids of whey is high and under favorable conditions these solids can effectively transfer milk flavor to foods. It is highly advantageous that whey protein will not coagulate at low temperatures in the presence of fruit acids and that it forms a soft, easily dispersible curd during the heat treatments used in cooking and canning (132).

*Foams and emulsifiers.* The foaming properties of whey protein have been studied and compared with those of egg albumin by Peter and Bell (90). They found that the stability of foams made from concentrated whey from which part of the lactose has been removed may be increased by neutralization or by the addition of small quantities of tannic acid, saponin, or bisulfites. Beeching and Severn (7) report the preparation of a heat-coagulable foaming agent by the neutralization and filtration of the mother liquor from lactose manufacture. Whey protein foams may be used in many food preparations. However, whey protein cannot be used in place of egg white in certain cakes and custards in which air must be incorporated by whipping and a firm structure set up by heat coagulation. A whey protein whip will not support other ingredients when coagulated by heat.

Sweetened condensed whey can be whipped in 4 minutes to a foam having 200 per cent overrun and a stability of 15 hours (92). This whip, which resembles marshmallow in appearance, is useful for toppings, icings, fruit whips and similar products.

A foaming material for use in non-alcoholic beverages has been prepared by dissolving whey protein in sufficient sodium hydroxide so that the solution has a pH value of 7.0, and then adding an edible acid, such as citric, to bring the reaction to pH 4.0-5.0 (33).

A mixture of 10 lb. of lipoid-free dried whey and 80 lb. of whole egg was found by Clickner (21) to have as good emulsifying properties as pure egg yolk and to be suitable for use in mayonnaise.

*Whey drinks.* These are made by adding highly flavored fruit or vegetable juices to whey. Whey adds to the nutritive value of a beverage, but it generally does not improve its flavor. A tomato-whey drink is made by mixing 65 per cent tomato juice, 0.4 per cent salt, and 34.6 per cent whey, including enough whey cream to give 2 per cent fat in the finished beverage (133). The reaction should be pH 4.4. The mixture is heated to 140° F., homogenized at 2,500 lb. pressure, canned, and sterilized by heating at 200° F. for 25 minutes. Other vegetable juices or fruit juices may be substituted for tomato juice. These beverages, when freshly mixed and promptly used, have flavor, color, and body superior to those of the canned and sterilized product.



A buttermilk type of beverage may be made by segregating the whey protein in a part of the whey (113). The whey from which the fat has been separated is boiled to coagulate the whey protein, the clear portion (five-sixths of the total) drawn off and discarded, and the remainder homogenized to redisperse the protein. The product contains about 4.1 per cent whey protein, 4.8 per cent lactose, and 0.5 per cent each of ash and fat. It should be possible to prepare a product of approximately this composition by means of high-speed centrifugal separation of boiled cheese whey, as discussed in the section on whey protein.

*Soups.* For immediate consumption soups can be prepared by using fresh whey in place of water. Soups that are to be canned and sterilized are made more easily with whey than with milk solids. A tomato soup containing whey solids retains the natural tomato acidity and does not contain clots or lumps of protein after heating. A formula for cream style tomato soup follows (132):

Whey solids, 4 per cent; milk fat, 4 per cent; flour or starch binder, 2.8 per cent; salt, 1 per cent; sugar, 1 per cent; fresh tomato juice, 70 per cent; added water, 17.2 per cent. Warm the mixture to 110° F., homogenize it at 2,500 lb., heat to 176° F., can, and sterilize by heating at 240° F. for 60 minutes without agitation.

*Cheese and cheese foods.* These may be divided into three classes: (a) whey cheese, (b) whey protein cheese, and (c) process cheese foods.

Whey cheese, known as mysost or primost, has been made for centuries in northern Europe, but the quantity produced in the United States is small. It is made by boiling the whey, generally in an open iron pan 8 to 10 feet in diameter. When it has the consistency of mortar, the hot, pasty mass is placed in tubs in which it is cooled and stirred in order to cause the lactose to form small crystals (27, 102, 122). Primost is packed into greased, wooden, cubical molds to cool and harden.

Albumin cheese was described in 1895 by Babcock (5). Ricotta or Ziger is produced from protein that has been removed from whey by one of the methods described earlier. Sammis (101) states that 5 to 10 per cent of skim or whole milk may be added to the whey before it is heated. The curd is placed in metal hoops, allowed to settle overnight, bandaged and pressed. The cheese may be salted and sold in fresh condition or dried at 110° F.

Whey protein curd recently has been converted into a Roquefort-type cheese (94). Four pounds of curd are recovered from 100 lb. of separated whey and, when pressed, the curd contains about 77 per cent moisture, 16.5 per cent protein, and 2.5 per cent fat. It is probable that whey protein curd can be converted into soft cheeses of other types or into a suitable base for cheese spreads.

Process cheese foods provide one of the largest uses for whey solids. The whey is added to the emulsified cheese mixture in the form of plain condensed or dried whey. Such mixtures contain at least 51 per cent cheese, less than 3 per cent emulsifying salts, organic acids to adjust the reaction to not less than pH 4.5, and a seasoning agent (24). A process cheese food may be made by mixing 93.5 per cent natural cheese, 5 per cent condensed whey containing 65 per cent solids, and 1.5 per cent emulsifying salts, heating with stirring at 165° F., and packaging hot (114).

*Bakery products.* Products containing whey solids are superior to those containing no milk products, but the products containing whey solids usually are considered inferior to those containing whole or skim milk concentrates. However, Davies (26) has stated that in England "... the use of dried whey in bread-making has proved time and again that the size of loaf, texture, taste, and general appeal of the bread are quite equal to that of milk bread." Any inferiority for this use of whey in comparison with milk is due principally to the relatively high salt and low protein of the whey solids, though the condition of the protein evidently is a factor. Concentrated whey products sometimes impart a salty, acid, or even a bitter taste to bread or cake. There is current interest in new types of whey concentrates developed especially for use in bakery products.

Since Greenbank *et al.* (39) showed that high heat treatment of milk contributed to improvement of bread containing it, whey protein has been suspected of playing an important role in bakery products containing milk. Studies on nitrogen distribution have shown that more than 90 per cent of the whey protein is coagulated when milk is heated above 200° F. for a few minutes (42, 70, 98, 99). This relationship between heat treatment and denaturation of the whey protein has prompted the suggestion that the determination of soluble or undenatured whey protein might be used as a test for the baking quality of dried skim milk (37, 41). In any case, heat treatment of whey destined for use in baked products appears highly desirable.

Processes have been patented for conditioning whey protein (86) and for separating it (54) for use in bread. A dry, comminuted, siftable, water-dispersible shortening composed of particles of fat coated with whey solids has been produced for use in prepared dry mixes and for other bakery purposes (19, 52).

Funder (36), in an extensive study, compared the volumes of water, whey and skim milk breads. For each 100 kg. of flour, he found volume increases over water bread of 6.2 kg. for fluid whey, 13.3 kg. for whey concentrated in the ratio of 2 to 1, and 9.5 kg. for plain skim milk. Several other workers have investigated the use of whey in bread (43, 56, 91, 121).

Whey may be used in a standard bread formula by adding either 3 to 4 lb. of whey solids (as fluid, plain condensed, or dried whey) or 7 to 10 lb. of sweetened condensed whey per 100 lb. of flour. The sugar and water in the formula should be adjusted to compensate for the addition of these ingredients in the whey.

Whey helps in producing a cake-like texture in sweet goods, and it may be added to conventional formulas for cakes and cookies (82, 136). A canned pudding has been developed in which as much as 22 per cent of the solids are whey solids (13).

*Candies.* Such types of candies as fudge, caramel and taffy can be made with a whey solids content of 14 to 40 per cent (55, 134, 135). Plain condensed, sweetened condensed, or dried whey may be used as a source of whey solids. Sweet rennet-type whey is preferred to neutralized acid whey because of its superior flavor. Whey is especially useful in fudge; the lactose on crystallizing contributes to the desired grainy texture. Whey caramels should be fortified with casein-containing milk solids in order to produce the characteristic chewy body. Whipped sweetened condensed whey may be used to incorporate air in special types of candy. A formula for whey fudge follows (134):

Sweetened condensed whey, 43 per cent; sugar, 11 per cent; corn sirup, 9 per cent; invert sirup, 3 per cent; butterfat, 2.5 per cent; chocolate, 6 per cent; fondant, 20 per cent; powdered lactose, 0.1 per cent; nuts (optional), 5.4 per cent; vanilla to flavor. Cook (with stirring) the condensed whey, sugar, invert sirup, and half the corn sirup. The butterfat is added as cream or butter after the sirup has been partly boiled down. Cook to 248° F. Cool 25 or 30° or transfer to smaller pouring kettles, add the remaining corn sirup, the fondant and chocolate, and stir well for several minutes. Add the powdered lactose, flavoring and nuts. Stir. Pour into wooden forms.

*Spirit vinegar.* It may be made from the alcohol produced by fermentation of whey by the procedure described earlier. A simple distillation of the fermented whey will yield a dilute alcohol suitable for conversion to vinegar. The dilute alcohol is allowed to trickle over beech shavings or birch twigs impregnated with the acetic-acid-producing organism. A current of air passing upward through the vinegar converter accelerates the fermentation.

*Food acidulant.* Colorless 50 per cent lactic acid is used as a food acidulant in sherbets and bottled beverages and as a preservative and firming agent for pickles. The production of edible lactic acid from crude acid is a highly technical chemical process (17).

*Whey butter.* This is made from whey cream in accordance with usual buttermaking procedures. The low ash and the absence of casein make



possible rapid churning. Hence, a butter of good body and texture can be produced by churning at a lower temperature than customarily is employed in churning cream from whole milk. The churning time of cream from whole milk can be shortened somewhat by adding whey cream.

#### *Pharmaceutical Uses*

Whey furnishes raw material for the preparation of several important pharmaceuticals, such as protein hydrolyzates and penicillin. Certain other products derived wholly or in part from whey and sold over the pharmaceutical counter in drug stores might properly be classed as foods, such as lactose and infant foods. The use of lactose as a basis for pills definitely is a pharmaceutical use.

*Lactose.* This often is added to infant foods based on cows' milk for the purpose of making the composition of the food more nearly like that of human milk. High grade technical or USP lactose is added to modified cows' milk in sufficient quantity so that it has a concentration of carbohydrate that may be as great as 52 per cent of the solids of the milk. Such infant foods generally are either dried or canned and sterilized. Lactose also is sold for use in feeding formulas prescribed for preparation in the home. Because the common or *alpha* lactose is only slowly soluble, it has been found more convenient to use the more rapidly soluble *beta* form of this sugar. The resulting solution is the equilibrium mixture of the two forms, whether *alpha* or *beta* lactose is used in making the solution.

*Riboflavin concentrates.* Riboflavin and other materials can be purified and concentrated by adsorption on lactose (63, 64, 84). By suitable choice of degree of super-saturation of the lactose, concentration of riboflavin, and working temperatures, adsorbates containing approximately 300  $\gamma$  of riboflavin per g. of lactose can be produced. It is possible to obtain even greater concentrations if excessive time is allowed for the crystallization of the sugar. The mother liquor of lactose manufacture may be used for the preparation of riboflavin adsorbate. After the removal of crude lactose that has crystallized at 140° F., the filtrate is cooled to between 40 and 50° F. and seeded with lactose crystals. A second crop of lactose crystallizes slowly over a period of 24 hours and contains about 100  $\gamma$  of riboflavin per g. of sugar. By recrystallization of this crude adsorbate, the concentration of riboflavin can be increased to about 300  $\gamma$  per g. of lactose.

*Penicillin.* Since 1943, several million lb. of lactose have been used each year as a component of the nutrient mixture in which penicillin is produced. Lactose acts to ensure higher yields than are obtained when it is not used, but it is not essential in the process. The possibilities of substitution

of other carbohydrates for lactose, of synthetic production of penicillin, and of the discovery of other antibiotics that will supersede penicillin make it seem unlikely that lactose long will be used for this purpose.

*Hydrolyzed lactose.* Lactose hydrolysis, which produces a mixture of glucose and galactose, offers the possibility of preparing a sweeter, more soluble form of carbohydrate from lactose for food use. Impure lactose, or the lactose in whey, when hydrolyzed by acid, yields a product which has a disagreeable taste and is contaminated with whey salts, humin and other products of protein hydrolysis.

Ramsdell and Webb (93) in hydrolyzing pure lactose found that there was a gradual destruction of glucose as it was formed. They found the optimal procedure to be as follows:

Heat 2,100 g. of pure lactose, 49 g. of N hydrochloric acid and 4,851 g. of water to 297° F. in a pressure kettle. Allow 60 minutes to reach 297° and hold 5 minutes. Add carbon black, filter, concentrate to 60 per cent solids, and adjust the reaction to pH 5.0.

Glucose and galactose to the amount of 93 per cent of the theoretical are obtained. The solubility of a mixture of equal parts of both is 42 per cent. The maximal concentration of a mixture of the two hexoses soluble at 77° F. is 58.3 per cent and consists of 49.8 per cent glucose and 8.5 per cent galactose. A process for making hydrolyzed lactose caramel has been described (79).

Enzymic hydrolysis of lactose in whey and other dairy products would be the simplest method of reducing the concentration of lactose and increasing the sweetness of the product. The use of an enzyme obtained from kefir grains to treat milk products to be used in ice cream has been suggested by Turnbow (127). However, no adequate source of a lactase has been available. Browne and Webb (14) investigated a number of possible sources, but were unable to obtain uniformly active lactase preparations suitable for commercial use. Current work in several laboratories, however, seems likely to result in the preparation of an enzyme of adequate strength in commercial quantities.

*Hydrolyzed protein.* Protein hydrolyzates usually are prepared by pharmaceutical companies, although several of the large dairy organizations have manufactured the hydrolyzate from whey protein. The flavor of the hydrolyzate derived from whey protein is said to be less objectionable than that derived from casein. Protein may be hydrolyzed by acid, by alkali or by enzymes. The reaction is complete when all the peptide linkages are broken. The rate of hydrolysis is that of a second-order reaction.

Sahyun (100) has reviewed the factors concerned with protein hydrolysis. Acid hydrolysis may be conducted with any of several acids, although

sulfuric generally is preferred. Tryptophane is destroyed during acid hydrolysis. The following directions for hydrolysis of protein by acid and by enzyme were given by Sahyun (100):

Two kilograms of protein is mixed with 14 l. of 5 N (25 per cent) sulfuric acid, the mixture autoclaved at 248° F. for 16 hours, limed to pH 10, filtered, and the residual calcium and sulfate ions removed. The amino acid preparation then is concentrated, and sterilized or dried.

There are numerous proteolytic enzymes capable of hydrolyzing proteins. Some of the amino acids are liberated sooner than others, but the degradation follows the general pattern: Protein → proteoses → peptones → peptides → amino acids.

To hydrolyze 100 g. of protein, it is mixed with 700 cc. of water, 10 cc. chloroform, 0.5 g. pancreas extract (trypsin), and sufficient 5 N (20 per cent) sodium hydroxide to produce a reaction of pH 8. The mixture is incubated under controlled conditions for 8 to 12 days, heated, filtered, concentrated and dried. (100)

When proteins are hydrolyzed by alkali, no humin is formed, as is the case in acid hydrolysis. However, the amino acids, with the exception of glycine, are racemized, which is objectionable, since not all of the racemic amino acids are utilized in animal metabolism.

*Therapeutic products.* Products consisting chiefly of the protein and mineral constituents of whey have been prepared by patented processes. One method is to precipitate the protein-mineral complex with alkali, separate it, wash or reprecipitate it to free it from any objectionable protein decomposition products, and finally dry it (95, 129). Another method is to precipitate electrolytically, filter, and wash and dry the precipitate. A product thus is obtained containing approximately 18 per cent calcium, 6 per cent phosphorus and 20 per cent protein (22). Another therapeutic product consists of whey, an edestin-calcium solution derived from hempseed, and magnesium sulfate (112).

#### *Chemical Uses*

*Lactic acid.* This is used industrially in leather manufacture (142). The highly colored, crude grades, marketed in 22 or 44 per cent concentration, are used in diluted condition to neutralize the lime in limed hides. The requirements for this purpose of a weak acid forming a soluble calcium salt are satisfied by lactic acid. Sodium lactate solutions resemble glycerol in consistency and are used as substitutes for it in textile printing and in paper-making. Ethyl, butyl and other lactate esters have use as solvents and plasticizers. Lactic esters can be used as starting materials for the production of the industrially important acrylates.



*Butyl alcohol.* This is a by-product of the fermentation producing riboflavin, and its esters are useful as solvents.

## REFERENCES

- (1) ALLEN, L. A., AND WATSON, S. J. Lait, 14: 889-895. 1934.
- (2) AMBROSE, A. S. U. S. Patent 1,991,189, Feb. 12, 1935.
- (3) ANONYMOUS. Food, 14(171): 319-322. 1945.
- (4) ARZBERGER, C. F. U. S. Patent 2,326,425, Aug. 10, 1943.
- (5) BABCOCK, S. M. Wis. Agr. Expt. Sta. Rept., 12: 134. 1895.
- (6) BEARDSLEE, A. C. U. S. Patent 2,336,461, Dec. 14, 1943.
- (7) BEECHING, E. I., AND SEVERN, G. W. C. British Patent 560,840, July 19, 1943.
- (8) BELL, R. W. Ind. Eng. Chem., 22: 51-55. 1930.
- (9) BELL, R. W., AND PETER, P. N. Ind. Eng. Chem., 20: 510-512. 1928.
- (10) BELL, R. W., PETER, P. N., AND JOHNSON, W. T., JR. J. Dairy Sci., 11: 163-174. 1928.
- (11) BERTRAM, K., AND LEMMERICH, E. U. S. Patent 2,335,380, Nov. 30, 1943.
- (12) BOSWORTH, A. W. U. S. Patent 1,246,858, Nov. 20, 1917.
- (13) BROWNE, H. H., AND WEBB, B. H. Food Inds., 13: 36-38, 96. Nov., 1941.
- (14) BROWNE, H. H., AND WEBB, B. H. Unpublished results. 1942.
- (15) BURKEY, L. A., AND WALTER, H. E. J. Dairy Sci., 29: 503. 1946.
- (16) BURKEY, L. A., AND WALTER, H. E. BDIM-Inf-46, Bur. Dairy Inds., U. S. Dept. Agr. 1947.
- (17) BURTON, L. V. Food Inds., 9: 571-575. 1937.
- (18) CARY, C. A., AND HARTMAN, A. M. Unpublished work.
- (19) CHAPIN, E. K. U. S. Patent 2,392,833, Jan. 15, 1946.
- (20) CHUCK, F. Y. U. S. Patent 2,016,592, Oct. 8, 1935.
- (21) CLICKNER, F. H. U. S. Patent 2,030,964, Feb. 18, 1936.
- (22) CLICKNER, F. H. U. S. Patent 2,076,400, April 6, 1937.
- (23) CLICKNER, F. H. U. S. Patent 2,091,629, Aug. 31, 1937.
- (24) COLLINS, M. Federal Register, 12: 1203-1204. Feb. 21, 1947.
- (25) DANIEL, L., AND NORRIS, L. C. Food Research, 9: 313-318. 1943.
- (26) DAVIES, W. L. Dairy Inds., 2: 213-215. June, 1937.
- (27) DOAN, C. F., AND MATHESON, K. J. U. S. Dept. Agr. Bull. 608. 1932.
- (28) DOOB, H., JR., WILLMANN, A., AND SHARP, P. F. Ind. Eng. Chem., 34: 1460-1468. 1942.
- (29) DUNHAM, H. V. U. S. Patent 709,003, Sept. 16, 1902.
- (30) ELDREDGE, E. E. U. S. Patent 1,923,427, Aug. 22, 1933.
- (31) ERSHOFF, B. H., AND DEUEL, H. J., JR. J. Nutrition, 28: 225-234. 1944.
- (32) FARRALL, A. W. Dairy Engineering. John Wiley & Sons, New York, N. Y. 1942.
- (33) FAULKNER, J. E. U. S. Patent 2,391,559, Dec. 25, 1945.
- (34) FLANIGAN, G. E., AND SUPPLEE, G. C. U. S. Patent 2,023,014, Dec. 3, 1935.
- (35) FRENCH, R. B., AND COWGILL, G. R. J. Nutrition, 14: 383-394. 1937.
- (36) FUNDER, L. Myse Og Skummet Melk Til Brodbakning. Statens Meieriforsok, Beretning Nr. 15. 1922.
- (37) GEDDES, W. F., AND JENNESS, R. Dairy Products Inst., Univ. Minn., Abs. Pp. 158-159. Sept., 1947.
- (38) GORDON, W. G. U. S. Patent 2,377,624, June 5, 1945.
- (39) GREENBANK, G. R., STEINBARGER, M. C., DEYSHER, E. F., AND HOLM, G. E. J. Dairy Sci., 10: 335-342. 1927.
- (40) HANDLER, P. J. J. Nutrition, 33: 221-234. 1947.
- (41) HARLAND, H. A., AND ASHWORTH, U. S. Food Research, 12: 247-251. 1941.

- (42) HARLAND, H. A., AND ASHWORTH, U. S. *J. Dairy Sci.*, 28: 879-886. 1945.
- (43) HENRY, K. M., HOUSTON, J., KON, S. K., POWELL, J., CARTER, R. H., AND HALTON, P. *J. Dairy Research*, 12: 184-212. 1941.
- (44) HOFFMANN, F. *Treatise on the Virtues and Uses of Whey*. L. Davis and C. Reymers. London, 1761.
- (45) HUIZENGA, S. *Centr. Zuckerind.*, 43(51): 1054-1056. 1935.
- (46) HUNZIKER, O. F. *Condensed Milk and Milk Powder*. 6th Ed. Published by the author at La Grange, Ill. 1946.
- (47) IRVINE, O. R., AND SPROULE, W. H. *Can. Dairy Ice Cream J.*, 19(3): 62-64. 1940.
- (48) JACK, E. L., AND WASSON, A. J. *J. Dairy Sci.*, 24: 85-92. 1941.
- (49) JOHNSON, A. H., WEISBERG, S. M., JOHNSON, J. J., AND PARKER, M. E. U. S. Patent 2,071,346, Feb. 23, 1937.
- (50) KAUFFMANN, W., AND VAN DER LEE, P. J. U. S. Patent 2,183,141, Dec. 12, 1939.
- (51) KON, S. K. *J. Dairy Research*, 9: 248-249. 1938.
- (52) KRAFT, G. H. U. S. Patent 2,035,899, March 31, 1936.
- (53) KRAFT, G. H. U. S. Patent 2,118,252, May 24, 1938.
- (54) KREMERS, K. U. S. Patent 2,349,969, May 30, 1944.
- (55) LANGWILL, K. E. *Mfg. Confectioner*, 21(8): 15-16. 1941.
- (56) LARSEN, L. *Tids. Norske Landbruk*, 40: 18-25. 1933.
- (57) LAVETT, C. O. U. S. Patent 2,143,019, Jan. 10, 1939.
- (58) LAVETT, C. O. U. S. Patent 2,172,393, Sept. 12, 1939.
- (59) LAVETT, C. O. U. S. Patent 2,188,907, Feb. 6, 1940.
- (60) LAVETT, C. O. U. S. Patent 2,197,804, April 23, 1940.
- (61) LAVETT, C. O. U. S. Patent 2,232,248, Feb. 18, 1941.
- (62) LEVITON, A. U. S. Patent 2,129,222, Sept. 6, 1938.
- (63) LEVITON, A. *Ind. Eng. Chem.*, 35: 589-593. 1943.
- (64) LEVITON, A. *Ind. Eng. Chem.*, 36: 744-745. 1944.
- (65) LEVITON, A. *J. Am. Chem. Soc.*, 68: 835-840. 1946.
- (66) LEVITON, A. In press.
- (67) LEVITON, A., AND LEIGHTON, A. *Ind. Eng. Chem.*, 30: 1305-1311. 1938.
- (68) LYMAN, J. F. U. S. Patent 1,954,769, April 10, 1934.
- (69) MEADE, R. E., POLLARD, H. L., AND RODGERS, N. E. U. S. Patent 2,369,680, Feb. 20, 1945.
- (70) MENEFEE, S. G., OVERMAN, O. R., AND TRACY, P. H. *J. Dairy Sci.*, 24: 953-968. 1941.
- (71) MEYER, E. M. U. S. Patent 1,787,754, Jan. 6, 1931.
- (72) MILLS, R., BREITER, H., KEMPSTER, E., McKEY, B., PICKENS, M., AND OUTHOUSE, JULIA. *J. Nutrition*, 20: 467-476. 1940.
- (73) MINER, C. S. U. S. Patent 2,202,161, May 25, 1940.
- (74) MORGAN, A. F., COOK, B. B., AND DAVISON, H. G. *J. Nutrition*, 15: 27-43. 1938.
- (75) MÜLLER, W. *Molkerei-Ztg. (Hildesheim)*, 53: 442. 1939.
- (76) MUNIN, F. *Fette u. Seifen*, 48: 144. 1941.
- (77) MYERS, R. P., AND WEISBERG, S. M. U. S. Patent 2,128,845, Aug. 30, 1938.
- (78) NABENHAUER, F. B. *Ind. Eng. Chem.*, 22: 54-56. 1930.
- (79) N. V. Lyemph Leeuwarder Ijs Melkproductenfabriek. Dutch Patent 57,374, April 15, 1946.
- (80) OKUDA, Y., AND ZOLLER, H. F. *J. Ind. Eng. Chem.*, 13: 515-519. 1921.
- (81) OUTHOUSE, JULIA. *Ill. Agr. Expt. Sta., Ann. Rept.*, 1934-1935: 293-294. 1936.
- (82) PEARCE, J. A. *Can. Dairy Ice Cream J.*, 26(1): 64, 66. 1947.
- (83) PEEBLES, D. D. U. S. Patent 1,976,246, Sept. 10, 1934.
- (84) PEEBLES, D. D. U. S. Patent 2,069,388, Feb. 2, 1937.

- (85) PEEBLES, D. D. U. S. Patent 2,126,807, Aug. 16, 1938.
- (86) PEEBLES, D. D. U. S. Patent 2,336,634, Dec. 14, 1943.
- (87) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 1,928,135, Sept. 26, 1933.
- (88) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 2,088,606, Aug. 3, 1937.
- (89) PEEBLES, D. D., AND MANNING, P. D. V. U. S. Patent 2,181,146, Nov. 28, 1939.
- (90) PETER, P. N., AND BELL, R. W. Ind. Eng. Chem., 22: 1124-1128. 1930.
- (91) PROCOPIO, M. Chim. ind. agr. biol., 19: 64-66. 1943.
- (92) RAMSDELL, G. A., AND WEBB, B. H. J. Dairy Sci., 21: 305-314. 1938.
- (93) RAMSDELL, G. A., AND WEBB, B. H. J. Dairy Sci., 28: 677-686. 1945.
- (94) REED, O. E. Rept. of the Chief, Bur. Dairy Ind., U. S. Dept. Agr. 1945.
- (95) RIGGS, L. K., AND CLICKNER, F. H. U. S. Patent 2,123,203, July 12, 1938.
- (96) ROGOSA, M. J. Bact., 45: 459-460. 1943.
- (97) ROGOSA, M., BROWNE, H. H., AND WHITTIER, E. O. J. Dairy Sci., 30: 263-269. 1947.
- (98) ROWLAND, S. J. J. Dairy Research, 5: 46-53. 1933.
- (99) ROWLAND, S. J. J. Dairy Research, 9: 42-46. 1938.
- (100) SAHYUN, M. Outline of the Amino Acids and Proteins. Chap. 4. Reinhold Pub. Corp., New York, N. Y. 1944.
- (101) SAMMIS, J. L. Cheese Making. 11th Ed. The Cheese Maker Book Co., Madison, Wis. Pp. 304-305. 1946.
- (102) SAMMIS, J. L. Cheese Making. 11th Ed. The Cheese Maker Book Co., Madison, Wis. Pp. 308-310. 1946.
- (103) SCHANTZ, E. J., ELVEHJEM, C. A., AND HART, E. B. J. Biol. Chem., 122: 381-390. 1938.
- (104) SCOTT, A. W. Hannah Dairy Research Institute, Bull. 4. 1932.
- (105) SCOTT, M. J., AND GRAHAM, R. W. New Zealand J. Agr., 38: 112-114. 1929.
- (106) SHARP, P. F. U. S. Patent 1,810,682, June 16, 1931.
- (107) SHARP, P. F. U. S. Patent 1,956,811, May 1, 1934.
- (108) SHARP, P. F., AND DOOB, H., JR. J. Dairy Sci., 24: 679-690. 1941.
- (109) SHARP, P. F., AND HAND, D. B. U. S. Patents 2,182,618 and 2,182,619, Dec. 5, 1939.
- (110) SHARP, P. F., AND TROY, H. C. J. Dairy Sci., 13: 140-157. 1930.
- (111) SIMMONS, N. L. U. S. Patent 1,763,633, June 10, 1933.
- (112) SIREK, J. U. S. Patent 2,023,359, Dec. 3, 1935.
- (113) SMILLIE, D. M., AND WIGHT, J. British Patent 548,836, Oct. 27, 1941.
- (114) SOMMER, H. H., AND TEMPLETON, H. L. Wis. Agr. Expt. Sta. Research Bull. 137. 1939.
- (115) SPELLACY, J. R. U. S. Patent 2,163,331, June 20, 1939.
- (116) STEFANIAK, J. J., RUPEL, I. W., BOHSTEDT, G., AND PETERSON, W. H. J. Dairy Sci., 30: 103-114. 1947.
- (117) STRINGER, W. E. Food Inds., 11: 72-74. 1939.
- (118) STRINGER, W. E. Food Inds., 11: 262-263, 290. 1939.
- (119) SUPPLEE, G. C. U. S. Patent 2,173,922, Sept. 26, 1939.
- (120) SUPPLEE, G. C., AND FLANIGAN, G. E. U. S. Patent 1,954,602, April 10, 1934.
- (121) SVENSON, J. Mehl u. Brot, 39: 357-360. 1939.
- (122) THOM, C., AND FISK, W. W. The Book of Cheese. MacMillan Co., New York, N. Y. 295. 1938.
- (123) THOMSEN, L. C. J. Dairy Sci., 19: 503. 1936.
- (124) TREBLER, H. A., AND HARDING, H. G. Chem. Eng. Progress, 43: 255-266. 1947.
- (125) TRIGGS, W. W. British Patent 434,058, Oct. 27, 1934.
- (126) TUFFET, E. Natl. Butter Cheese J., 32(8): 11. 1941.
- (127) TURNBOW, G. D. U. S. Patent 1,737,101, Nov. 26, 1929.



- (128) WAITE, R. J. Dairy Research, 12: 71-77. 1941.
- (129) WANSHENK, O. B. U. S. Patent 2,123,218, July 12, 1938.
- (130) WASHBURN, R. M. U. S. Patent 1,870,270, Aug. 9, 1932.
- (131) WATSON, P. D. Ind. Eng. Chem., 26: 640-645. 1934.
- (132) WEBB, B. H. Food Research, 3: 233-238. 1938.
- (133) WEBB, B. H. BDIM 847, Bur. Dairy Ind., U. S. Dept. Agr. 1939.
- (134) WEBB, B. H. Mfg. Confectioner, 21(3): 14-16. 1941.
- (135) WEBB, B. H., AND HUFNAGEL, C. F. Food Research, 5: 185-195. 1940.
- (136) WEBB, B. H., AND HUFNAGEL, C. F. Natl. Butter Cheese J., 37(12): 34, 35, 68, 70, 72. 1946.
- (137) WEBB, B. H., AND RAMSDELL, G. A. U. S. Patent 2,119,614, June 7, 1938.
- (138) WEBB, B. H., AND RAMSDELL, G. A. Natl. Butter Cheese J., 35(7): 18-24; 35(8): 18. 1944.
- (139) WEIMAR, A. C. U. S. Patent 1,381,605, June 14, 1921.
- (140) WEISBERG, S. M., CHAPPELL, F. L., STRINGER, W. E., AND STEVENS, S. U. S. Patent 2,071,368, Feb. 23, 1937.
- (141) WHITTIER, E. O., CARY, C. A., AND ELLIS, N. R. J. Nutrition, 9: 521-532. 1935.
- (142) WHITTIER, E. O. J. Dairy Sci., 27: 505-537. 1944.
- (143) WILSON, C. W. U. S. Patent 1,975,998, Oct. 9, 1934.
- (144) YAMASAKI, I. U. S. Patent 2,297,671, Sept. 25, 1942.

# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

MARCH, 1948

NUMBER 3

## EFFECT OF SEASON, BREED AND SPECIES OF RUMINANTS ON THE VITAMIN A POTENCY OF BUTTERFAT

B. C. RAY SARKAR<sup>1</sup>

*Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar, U. P., India*

Under European and American conditions, the vitamin A potency of butter from cows generally is found to be maximum in summer and minimum in winter (3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 19). It also has been reported that the variation in potency is influenced but little by the stage of lactation (6, 9, 18). Since Indian climate and feeding practices vary markedly from those in Europe and America, a study of the seasonal variation in vitamin A and some of the other constituents of milk and butterfat was considered desirable.

In previous reports from this laboratory (16, 17) the effect of various levels of carotene ingestion on the vitamin A potency of milk and butterfat of Haryana cows was studied. In order to make a comparative study of the vitamin A potency of butterfat from different breeds and from other species of ruminants, further work subsequently was carried out and some of the results are presented in this paper.

### EXPERIMENTAL PROCEDURE

The Institute dairy herd consisting of from 23 to 33 Haryana animals received different types of roughages, depending upon the season of the year, and a concentrate mixture (wheat bran 40 parts, gram husk 20 parts, ground nut cake 20 parts, rape cake 10 parts, and gram chuni 10 parts) at the rate of 1.5 lb. for maintenance and 1 lb. for each 2 lb. of milk produced. The animals, in addition, received 1 oz. of iodized salt and 1 oz. of bone meal per head daily.

The investigation lasted from April 1, 1941, to July 15, 1942, during which time fortnightly analyses were made to determine the carotene content of the feeds and the fat, carotene and vitamin A contents of the composite herd milk sample collected over a 3-day period from two milkings.

Received for publication October 20, 1947.

<sup>1</sup> Present address, Visiting Professor in the Department of Agricultural Chemistry, Michigan State College.

a day. The milk samples were analyzed also for total solids, protein, ash, calcium and phosphorus from the middle of November, 1941, until the conclusion of the experiment. Butter samples were prepared during typical drought periods (May and December) and green-feeding periods (February and July) in both winter and summer and analyzed for the Reichert-Meissl, Polenske, saponification and iodine values. The methods of analysis were the same as those previously reported (16).

To study the influence of breed and species, short-time experiments were conducted with Sahiwal heifers, goats and water buffaloes. Three Sahiwal first-calf heifers, each weighing approximately 688 lb., were fed berseem *ad libitum* in addition to the regular dairy grain mixture for a period of 19 days. The average consumption of berseem was 59 lb. and that of carotene was 1,009 mg. per day. Four goats, each having an average body weight of 67 lb., received berseem in addition to a concentrate for 18 days. The average daily consumption of berseem was 3,292 g. and the ingestion of carotene amounted to 108 mg. Two water buffaloes, each weighing approximately 865 lb., received 50 lb. of berseem daily. The level of feeding was not *ad libitum*, owing to the shortage of berseem, and the experimental period was only 11 days. The daily consumption of carotene was 785 mg. per animal. All of the milk samples used in this work were collected from two milkings a day during the last 3 days of the experimental period and analyzed for carotene, vitamin A, fat, solids-not-fat, protein and ash.

In calculating the total vitamin A potency of the butterfat, 0.6  $\mu$ g. of the carotene and 0.25  $\mu$ g. of the vitamin A were each taken as equal to 1 international unit of vitamin A (1).

#### RESULTS

*Variations in vitamin A potency of butterfat.* Table 1 presents the composite data for carotene, vitamin A, and total vitamin A potency of butterfat for each month of the experiment and the level of carotene ingestion for each corresponding month. In general, the maximum total vitamin A potency was reached during July, August and September (24,734 I.U. per lb.) and then diminished gradually through January. The average minimum value (16,093 I.U.) was obtained in November, December and January and then the potency began to rise, reaching the maximum value in March (24,861 I.U.). During April, May and June, the potency declined rapidly but in July the potency increased markedly. The maximum increase in total vitamin A potency was 55 per cent during the experimental period. The variations observed in total vitamin A potency were not due entirely to carotene activity or vitamin A activity alone but to a combination of both. The periodic fluctuations in carotene and vitamin A in butterfat can be correlated with the level of carotene ingestion subsequent to changing the type of fodders. On the microgram basis, the maximum and



minimum values reported in this paper are in agreement with those previously reported by Ray Sarkar and Sen (16) in experiments with cows under intensive green-feeding conditions. From the data on carotene ingestion (table 1) it has been calculated that a daily intake of about 45 lb. of average green fodder per cow will maintain the maximum vitamin A potency throughout the year (16).

TABLE 1  
*Seasonal variation in the vitamin A potency of butterfat of Hariana cows*

Month	Av. daily carotene intake per cow	Vitamin A potency of butterfat		
		Carotene	Vitamin A	Total
(1941)	(I.U.)	(I.U. per lb.)		
April .....	541,800	1,582	15,155	16,737
May .....	521,400	1,823	16,281	18,104
June .....	695,500	2,291	18,844	21,135
July .....	990,100	2,483	22,259	24,742
Aug. ....	1,495,300	2,712	22,126	24,838
Sept. ....	1,369,600	2,833	21,790	24,623
Oct. ....	507,000	2,389	18,486	20,875
Nov. ....	260,200	1,790	14,210	16,000
Dec. ....	210,700	908	15,301	16,209
(1942)				
Jan. ....	450,400	1,269	14,802	16,071
Feb. ....	1,268,500	2,671	19,786	22,457
March ....	850,400	2,814	22,047	24,861
April ....	379,500	1,793	19,315	21,108
May ....	154,800	1,375	16,386	17,761
June ....	139,800	1,378	16,286	17,664
July ....	1,625,400	2,756	18,770	21,526

Table 2 shows the effects of carotene-poor and carotene-rich rations on the carotene and vitamin A contents and on the total vitamin A potency of butterfat of cows at different stages of lactation. The vitamin A potency of butterfat in the post-colostral period remains practically unchanged with the progress of lactation, provided the same level of carotene ingestion is maintained throughout the lactation period. This observation is in conformity with that of Treichler *et al.* (18), Gillam *et al.* (9), and Brown *et al.* (6). These results indicate that the stage of lactation has little effect on the seasonal variation in the vitamin A potency of butterfat.

*Seasonal effect on the quality of butterfat.* A limited number of chemical constants were determined on the butterfat samples prepared at different seasons of the year. These results are summarized in table 3. The data indicate that within the same season, the iodine values increase when green fodder is included in the ration (samples 2 and 4), whereas the Polenske values increase during the summer months (samples 3 and 4). Apparently neither the feeding of green fodder nor season had any appreciable effect on the Reichert-Meissl or saponification values. The Reichert-Meissl num-

ber, however, seems to be significantly lower and the iodine number significantly higher than that reported for western butterfat. The Polenske value tends to be lower but the saponification number is the same as that reported for western butterfat. These observations, however, are tentative and must be confirmed by future experiments.

TABLE 2  
*Effect of stage of lactation on carotene and vitamin A contents of the butterfat of Haryana cows*

No. of cows	Month of lactation	Av. daily milk yield	Av. fat	Vitamin A potency of butterfat		
				Carotene	Vitamin A	Total
		(lb.)	(%)	(I. U. per lb.)		
Cows receiving carotene-poor rations						
5	1	12.0	5.2	785	14,876	15,661
2	2	12.5	4.8	708	14,876	15,584
5	3	11.4	4.8	785	15,242	16,027
2	4	15.5	4.7	729	14,144	14,873
3	6	9.7	5.2	652	15,775	16,427
1	7	4.0	4.5	776	15,232	16,008
	Weighted av.			748	15,066	15,814
Cows receiving carotene-rich rations						
5	3	11.8	4.5	2,712	21,660	24,372
4	4	10.3	4.5	2,349	21,717	24,066
5	5	9.0	4.4	2,590	21,548	24,138
4	6	7.5	4.9	2,692	21,484	24,176
2	7	9.0	4.9	2,481	21,412	23,893
1	8	9.0	4.5	2,242	21,791	24,033
	Weighted av.			2,566	21,594	24,160

*Variations in other milk constituents.* The variations in the quality of milk at various seasons as measured by differences in some of the main constituents are shown in table 4. The maximum, minimum, and average values obtained during the experimental period were 5.6, 4.5, and 4.9 per

TABLE 3  
*Chemical constants of the butterfat from herd milk at different seasons*

Sample no.	Month	Reichert-Meissl value	Polenske value	Saponification value	Iodine value
1	Dec.	22.0	1.1	226.0	36.0
2	Feb.	23.5	1.6	226.2	40.0
3	May	24.0	2.2	227.0	37.0
4	July	23.4	2.2	226.8	39.3

cent for fat; 8.97, 8.62, and 8.75 per cent for solids-not-fat; 3.70, 3.43, and 3.53 per cent for protein; 0.808, 0.729, and 0.771 per cent for ash; 0.150, 0.125, and 0.138 per cent for calcium; and 0.107, 0.091, and 0.100 per cent for phosphorus, respectively. However, the mean fat percentage for the

15.5-month experimental period was 4.8. The fat content tended to remain at a higher level during November, December and January, when a seasonal decline in milk production occurs due to the marked drop in temperature.

TABLE 4  
*Composition of herd milk at different seasons of the year (Haryana cows)*

Period	Fat	S.N.F.	Protein	Ash	Ca	P
(1941)	(%)	(%)	(%)	(%)	(%)	(%)
Nov. 16-30 .....	5.6	8.78	3.50	0.788	0.150	0.104
Dec. 1-15 .....	5.4	8.68	3.45	0.729	0.143	0.096
16-31 .....	5.1	8.87	3.43	0.793	0.138	0.098
(1942)						
Jan. 1-15 .....	5.2	8.97	3.70	0.778	0.138	0.103
16-31 .....	5.2	8.84	3.61	0.778	0.148	0.097
Feb. 1-15 .....	4.8	8.70	3.56	0.780	0.140	0.107
16-28 .....	4.7	8.62	3.48	0.769	0.133	0.105
March 1-15 .....	4.8	8.77	3.60	0.780	0.143	0.105
16-31 .....	4.5	8.78	3.52	0.769	0.143	0.107
April 1-15 .....	4.6	8.70	3.45	0.760	0.140	0.100
16-30 .....	5.0	8.64	3.48	0.808	0.145	0.099
May 1-15 .....	4.7	8.78	3.50	0.769	0.128	0.091
16-31 .....	4.8	8.64	3.48	0.760	0.125	0.093
June 1-15 .....	5.0	8.73	3.52	0.740	0.143	0.105
16-30 .....	4.7	8.70	3.47	0.788	0.140	0.098
July 1-15 .....	5.0	8.85	3.60	0.770	0.129	0.102
16-31 .....	4.7	8.72	3.67	0.747	0.128	0.091
Av. ....	4.9	8.75	3.53	0.771	0.138	0.100

There also were differences of 20.0 and 17.6 per cent, respectively, between the lowest and highest calcium and phosphorus values. Apart from the relatively wide percentage differences in fat, calcium and phosphorus, the changes in the other constituents were slight.

TABLE 5  
*The various constituents in milk and the carotene and vitamin A contents of butterfat from heifers, goats and buffaloes*

	Heifers	Goats	Buffaloes
Milk yield .....	10.6 lb.	383.5 ml.	16.2 lb.
Fat (%) .....	6.5	5.1	7.9
S.N.F. (%) .....	8.59	9.28	9.90
Protein (%) .....	3.73	4.54	4.03
Ash (%) .....	0.750	0.897	0.856
Carotene (I.U.) <sup>a</sup> .....	2,782	Trace	Trace
Vitamin A (I.U.) <sup>a</sup> .....	23,050	26,498	17,879
Total potency (I.U.) <sup>a</sup> .....	25,832		

<sup>a</sup> Per lb. of butterfat.

*Constituents in milk and butterfat of heifers, goats and buffaloes.* The data pertaining to the amounts of various constituents in milk and the carotene and vitamin A in butterfat from the three species are shown in table 5. Although the number of animals is small, it may be said that the



various species do differ in regard to the secretion of the various constituents in milk. This observation is not new. The interesting fact is the trace of carotene in the butterfat from goats and buffaloes, an amount which is not measurable quantitatively by the usual colorimetric method. Considerable individual variation in the vitamin A content also was noted in the case of each species. So far as total vitamin A potency of butterfat is concerned, goats and cows are almost equally efficient. It was observed subsequently in the case of the goats that the same potency could be attained even at a level of 60 per cent of the original carotene ingestion. The vitamin A potency of the butterfat of the buffalo was comparatively low. A true comparison could not be made because the level of carotene ingestion was not high enough owing to the shortage of green fodder. Subsequent studies with other animals under heavy green-fodder feeding have shown, however, that buffalo butterfat might contain as much as 20,480 I.U. per lb. as compared to 25,000-26,000 I.U. in the case of butterfat from cows and goats.

A comparison of the figures previously obtained for carotene and vitamin A in the butterfat from Haryana cows shows that the vitamin A potency is the same for the Sahiwal and Haryana breeds (16) when calculated on the same basis. The concentration of vitamin A in the butterfat of cows so far examined compares fairly well with that found for some western breeds (2, 4, 9), but the picture is different with respect to carotene. The two Indian breeds studied so far definitely secrete less carotene in butterfat. It is difficult to say without further experimentation where this physiological difference lies. In view of the lack of knowledge regarding the fate of carotene in the rumen, much importance has not been given to the value of about 70 per cent apparent fecal excretion in the case of cows and goats, as determined incidentally. If the rest of the carotene were absorbed, then the recovery of absorbed carotene as carotene and vitamin A in milk would amount to about 3.5 per cent. This value is practically the same for the heifers and goats under comparable feeding conditions. There is very little carotene in the plasma of goats as compared to 0.891 mg. per cent in the plasma of the Sahiwal heifers. On the other hand, the blood plasma levels of vitamin A were 0.170 mg. and 0.157 mg. per cent, respectively, for heifers and for goats. Buffalo blood was not examined in this connection. Goats and buffaloes possibly convert more of the absorbed carotene into vitamin A, which is readily transmitted into the milk.

#### SUMMARY

The seasonal variations in vitamin A potency of butterfat and other constituents in herd milk of Haryana cows have been investigated. The vitamin A potency varied with the level of carotene intake. The potency was maximal in the monsoon periods (July, August and September) when the cows were getting sufficient carotene from grazing and again during the

winter months (February and March) when large quantities of cultivated fodders were available. The average maximum total potency approximated 24,972 I.U. per lb., of which 2,700 I.U. were due to carotene. The average minimum potency of 16,093 I.U., of which 1,322 I.U. were due to carotene, was obtained in November, December and January, when very little green feed was available. The maximum variations in carotene, vitamin A, and total vitamin A potency were 212.0, 56.6, and 55.0 per cent, respectively. The vitamin A potency was not influenced by the stage of lactation.

Except for fat content, which was about 25 per cent higher in November, December and January, and for calcium and phosphorus, the solids-not-fat, protein and ash content of the milk remained unchanged throughout the experimental period.

The Polenske values of the butterfat were higher in summer than in winter but the Reichert-Meissl and saponification values did not show any seasonal change. The saponification value was the same as that reported in the literature for American butterfat, but the Reichert-Meissl number was approximately 20 per cent lower and the iodine number was about 10 per cent higher. The inclusion of green feed in the ration tended to increase the iodine value.

There was practically no difference in the carotene and vitamin A contents of butterfat from the cows of the Hariana and Sahiwal breeds. Although butterfat from goats contained only traces of carotene, the vitamin A content was as high as the total potency in the butterfat from cows. Buffalo butterfat examined in this investigation resembled that of goats in respect to carotene, but the vitamin A content was comparatively low.

The author is indebted to Dr. K. C. Sen for generously supplying all of the facilities needed in the course of this investigation. Further acknowledgment is made to Professor C. W. Duncan, Department of Agricultural Chemistry, Michigan State College, for many helpful suggestions in the preparation of this manuscript for publication.

#### REFERENCES

- (1) ANONYMOUS. Vitamin A in Butter. U. S. Dept. Agr. Misc. Pub. 571. 1945.
- (2) ATKESON, F. W., HUGHES, J. S., KUNERTH, B. L., PETERSON, W. J., AND KRAMER, M. Recovery of Carotene and Vitamin A from Butter when Cows Were Fed Unlimited Quantities of Green Rye. *J. Nutrition*, **14**: 621-629. 1937.
- (3) BAUMANN, C. A., AND STEENBOCK, H. Fat-soluble Vitamins. XXXVI. The Carotene and Vitamin A Contents of Butter. *J. Biol. Chem.*, **101**: 547-560. 1933.
- (4) BAUMANN, C. A., STEENBOCK, H., BEESON, W. M., AND RUPEL, I. W. Fat-soluble Vitamins. XXXIX. The Influence of Breed and Diet of Cows on the Carotene and Vitamin A Contents of Butter. *J. Biol. Chem.*, **105**: 167-176. 1934.
- (5) BERL, S., AND PETERSON, W. H. Determination and Content of Carotene and Vitamin A in Wisconsin Butter. *J. Nutrition*, **26**: 527-538. 1943.

- (6) BROWN, W. C., VANLANDINGHAM, A. H., AND WEARLEY, C. E., JR. Oxidized Flavor in Milk. IX. The Effect of the Quality of Hay and Early Stage of Lactation on the Carotene Content of Butterfat and on the Ascorbic Acid Content of the Milk and Their Relationship to the Development of Metal-induced Oxidized Flavor. *J. Dairy Sci.*, 24: 925-935. 1941.
- (7) DORNBUSH, A. C., PETERSON, W. H., AND OLSON, F. R. The Carotene and Vitamin A Contents of Market Milks. *J. Am. Med. Assoc.*, 114: 1748-1751. 1940.
- (8) ELLENBERGER, H. A., GUERRANT, N. B., AND FARDIG, O. B. Seasonal Variations in the Vitamin A and the Carotene Content of Retail Butters. *J. Nutrition*, 33: 39-52. 1947.
- (9) GILLAM, A. E., HEILBRON, I. M., FERGUSON, W. S., AND WATSON, S. J. Variations in the Carotene and Vitamin A Values of the Milk Fat (Butter) of Cattle of Typical English Breeds. *Biochem J.*, 30: 1728-1734. 1936.
- (10) GUGGENHEIM, K. Seasonal Fluctuations of the Vitamin A and C Contents of Palestinian Milks. *J. Hyg.*, 40: 690-698. 1940.
- (11) JENNESS, R., AND PALMER, L. S. The Vitamin A Potency of Creamery Butter Produced in Minnesota. *J. Dairy Sci.*, 28: 473-493. 1945.
- (12) KOEHN, C. J. Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama. *J. Dairy Sci.*, 26: 673-681. 1943.
- (13) LORD, J. W. Seasonal Variation of Carotene and Vitamin A in Butterfat and in Serum. *Biochem. J.*, 39: 372-374. 1945.
- (14) MORGAN, R. S., AND PRITCHARD, H. Average Vitamin A and Vitamin D Potency of Butter. *Analyst*, 62: 354-362. 1937.
- (15) PARRISH, D. B., MARTIN, W. H., ATKESON, F. W., AND HUGHES, J. S. The Vitamin A and Carotene Content of Market Butter Produced in Kansas. *J. Dairy Sci.*, 29: 91-99. 1946.
- (16) RAY SARKAR, B. C., AND SEN, K. C. The Chemical Composition and Vitamin A Potency of Cow Milk and Butterfat under Intensive Green Feeding and the Carotene Metabolism of Dairy Animals. *Indian J. Vet. Sci.*, 13: 219-227. 1943.
- (17) SEN, K. C., AND RAY SARKAR, B. C. The Vitamin A Potency of Cow Milk at Different Levels of Carotene Ingestion. *Indian J. Vet. Sci.*, 12: 179-189. 1942.
- (18) TREICHLER, R., GRIMES, M. A., AND FRAPS, G. S. Relation of the Color and Carotene Content of Butterfat to Its Vitamin A Potency. *Tex. Agr. Expt. Sta. Bull.* 513. 1935.
- (19) WILKINSON, H. The Vitamin A and Vitamin D Contents of Butter. II. Seasonal Variation. *Analyst*, 64: 17-23. 1939.



## PARTURIENT PARESIS. II. THE EFFECT OF PARTIAL VERSUS COMPLETE MILKING UPON THE TOTAL BLOOD SERUM CALCIUM OF DAIRY COWS AT PARTURITION<sup>1</sup>

VEARL R. SMITH AND R. P. NIEDERMEIER

*Department of Dairy Husbandry*

AND

R. G. HANSEN

*Department of Biochemistry, University of Wisconsin, Madison*

A common practice among dairymen is to partially milk cows for a few days following parturition, because it is believed to reduce the incidence of parturient paresis or milk fever. Several workers (2, 3, 6) have demonstrated that air inflation of the udders of cows with parturient paresis results in an increase in the total serum calcium and generally brings about recovery. When air pressures equal to 25 to 40 mm. Hg were maintained in the udder, inhibition of milk secretion was almost complete (4, 5). The efficacy of the air inflation treatment for parturient paresis has been attributed to resorption of milk or cessation of milk secretion caused by increased intramammary pressure, thereby preventing a further uptake of calcium by the mammary gland. As a result of these studies, the belief has become prevalent that the incidence of parturient paresis could be lowered if some milk was left in the udder to maintain pressure. This study was undertaken to learn the effect of complete milking immediately following parturition upon the incidence of parturient paresis and total blood serum calcium.

### EXPERIMENTAL PROCEDURE

Cows used were of the Holstein, Guernsey and Jersey breeds. They were divided into two groups by alternating cows within each breed. The cows in the partially milked group were managed in the conventional manner by permitting the calves to remain with the cows for 3 days following calving. About two-thirds of the milk was removed from the udders of this group beginning the day after calving. Complete milking began the fourth day subsequent to calving.

Calves were removed from the dams in the completely milked group before nursing, and the cows were milked completely with the aid of an intravenous injection of 10 I.U. of oxytocin from 1 to 3 hours after calving. The cows then went on the regular two-time milking schedule and were in-

Received for publication October 27, 1947.

<sup>1</sup>Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1  
Daily levels of total blood serum before and after parturition

Cow no.	Total blood serum calcium—mg. %												Remarks
	Days prepartum					Day of parturition	Days postpartum					Date of parturition	
	5	4	3	2	1		1	2	3	4	5		
Jerseys (completely milked group)													
711	10.1	11.2	10.9	10.2	10.9	9.9	10.9	9.5	10.4	10.0	10.3	6-13-45	Mild symptoms of milk fever
703	9.5	9.4	9.8	9.3	9.7	9.6	10.6	8.1	9.5	8.7	9.5	6-23-45	
667		9.6	9.8	9.9	9.8	5.9	9.7	10.6	10.3	10.3	—	6-27-45	
713			9.4	9.9	8.1	7.6	8.1	9.7	10.2	10.3	10.4	8-25-45	
B61			9.8	9.9	9.5	5.0	9.5	5.8	9.0	9.5	9.3	2-22-46	Mild symptoms of milk fever
B58				9.9	9.1	4.0	9.1	3.7	7.9	9.9	10.1	3-21-46	Milk fever on 3-21-46
705			10.0	9.9	9.8	6.8	9.8	6.3	7.0	9.4	9.9	8-3-46	
696	10.2	9.9	9.9	10.4	10.2	6.1	10.2	4.1	9.0	9.5	10.2	9-15-46	Milk fever on 9-16-46
731		10.7	10.7	10.4	10.1	9.7	10.1	10.4	10.6	—	—	9-23-46	
729	10.7	10.8	10.8	10.0	10.5	9.7	10.5	10.1	10.8	—	—	11-22-46	
B57	9.8	10.4	10.2	10.1	—	8.4b	10.9	7.6	8.0	9.6	8.9	4-14-47	Milk fever on 4-14-47
Jerseys (partially milked group)													
705	9.9	10.2	10.1	10.4	11.3	8.2	11.3	4.2	8.2	9.3	10.3	6-26-45	Milk fever on 6-27-45
671	10.0	10.6	10.2	10.2	9.3	7.4	9.3	4.7	14.4b	—	—	8-20-45	Milk fever 8-21-45, died 8-23-45
690			11.3	10.3	10.6	4.4	10.6	6.6	11.4	11.6	—	10-5-45	Milk fever on 10-5-45
731	10.5	9.2	11.6	10.6	10.9	10.3	10.9	10.3	10.6	10.6	10.4	10-22-45	
B57					9.3	6.0	9.3	4.1	10.4	11.3	11.6	12-2-45	Milk fever on 12-3-45
B63					9.2	4.7	10.8	7.8	11.2	10.9	—	3-24-46	Milk fever on 3-24-46
711	9.7	9.7	10.2	9.8	9.8	8.4	9.8	7.6	10.3	10.9	10.7	8-8-46	
713				9.8	9.8	—	9.8	8.7	5.9	6.1	7.9	8-31-46	Milk fever 8-31-46, died 9-18-46
703	10.2	10.7	10.9	10.7	8.5	4.0	8.5	8.0	10.4	10.6	10.2	9-15-46	Milk fever on 9-15-46
720				10.5	8.4	6.7	10.5	9.6	10.7	10.7	—	12-9-46	
B61			10.4	10.8	10.3	4.9	10.3	6.1	10.8	10.2	10.0	4-10-47	Milk fever on 4-10-47

<sup>a</sup> Either blood sample was not drawn or was destroyed accidentally.

<sup>b</sup> Treated for milk fever before blood sample was taken.

jected intravenously with oxytocin at each milking for 5 days subsequent to calving.

TABLE 2  
Average total blood serum calcium

	Total blood serum calcium—mg. %											Remarks
	Days prepartum					Day of parturition	Days postpartum					
	5	4	3	2	1		1	2	3	4	5	
<i>Jerseys</i>												
Completely milked group												
Av. 11 cows .....	10.1	10.2	10.2	10.0	9.9	7.5	7.6	8.6	9.4	9.7	9.8	3 cases milk fever
Partially milked group												
Av. 11 cows .....	10.1	10.1	10.7	10.4	9.8	6.5	7.1	9.1	10.3	10.2	10.2	8 cases milk fever
Both groups												
Av. 22 cows .....	10.1	10.2	10.4	10.2	9.8	7.0	7.3	8.8	9.8	10.0	10.0	11 cases milk fever
<i>Guernseys</i>												
Completely milked group												
Av. 9 cows .....	10.3	10.3	10.2	10.4	10.1	8.8	9.2	9.7	9.6	10.0	10.1	.....
Partially milked group												
Av. 11 cows .....	9.9	10.4	10.2	10.0	9.9	9.0	9.3	9.4	9.9	9.8	10.0	.....
Both groups												
Av. 20 cows .....	10.1	10.3	10.2	10.2	10.0	8.9	9.3	9.5	9.8	9.9	10.0	.....
<i>Holsteins</i>												
Completely milked group												
Av. 7 cows .....	10.1	10.5	10.4	10.5	9.8	9.0	8.4	9.3	10.1	9.8	10.3	.....
Partially milked group												
Av. 7 cows .....	11.1	10.2	10.5	10.3	10.1	9.5	9.7	9.9	9.9	10.2	9.9	.....
Both groups												
Av. 14 cows .....	10.4	10.4	10.5	10.4	10.0	9.2	9.1	9.6	10.0	10.0	10.1	.....
<i>Summary of all breeds</i>												
Av. 27 completely milked cows .....	10.2	10.3	10.2	10.3	9.9	8.3	8.4	9.1	9.6	9.8	10.0	3 cases milk fever
Av. 29 partially milked cows .....	10.2	10.2	10.4	10.2	9.9	8.2	8.5	9.4	10.1	10.1	10.0	8 cases milk fever

Venous blood samples were taken daily for 5 days previous to the anticipated day of parturition and for the first 5 days subsequent to calving. Blood samples were drawn within an hour of the same time daily. Total



blood serum calcium was determined by a modification of the Clark-Collip method (1).

The experiment ran from June, 1945, until May, 1947, and included a total of 56 cows, of which 29 were in the partially milked group and 27 in the completely milked group. All cows of the three breeds mentioned freshening in the experimental herd were included, with the exception of first-calf heifers, which rarely if ever have parturient paresis.

A case was not diagnosed as parturient paresis or milk fever unless the cow was "down" or in a coma with typical symptoms. Two cows in the completely milked group showed mild symptoms of milk fever but never went "down" and recovered without treatment.

#### RESULTS

Data for the Jerseys of both groups are presented in table 1. The average figures of total blood serum calcium for both groups of all breeds, as well as a summary of all breeds, are presented in table 2. The complete data for the other breeds are not presented, as all cases of milk fever occurred within the Jersey breed. The over-all incidence of the disease was 19.6 per cent, with eight cases of milk fever occurring in the partially milked group and three cases in the completely milked group. The Jersey breed had an incidence of 50 per cent, with 27.3 per cent in the completely milked group and 72.7 per cent in the partially milked group. Two of the Jersey cows (671 and 713, while in the partially milked group) died, although calcium treatment was administered. Cow no. 713 developed a case of severe ketosis, for which treatment proved ineffective, and this was the ultimate cause of her death.

Total blood serum calcium levels exhibited a characteristic drop on the day of calving, with a gradual return to normal by the fourth or fifth day subsequent to calving. The greatest drop was evident in the Jersey breed, with little difference in the averages of the Holstein and Guernsey breeds. The total blood serum calcium levels of the two groups closely parallel each other.

#### SUMMARY

The complete milking of cows immediately following parturition did not increase the incidence of parturient paresis or was the average total serum calcium greatly different for the two groups.

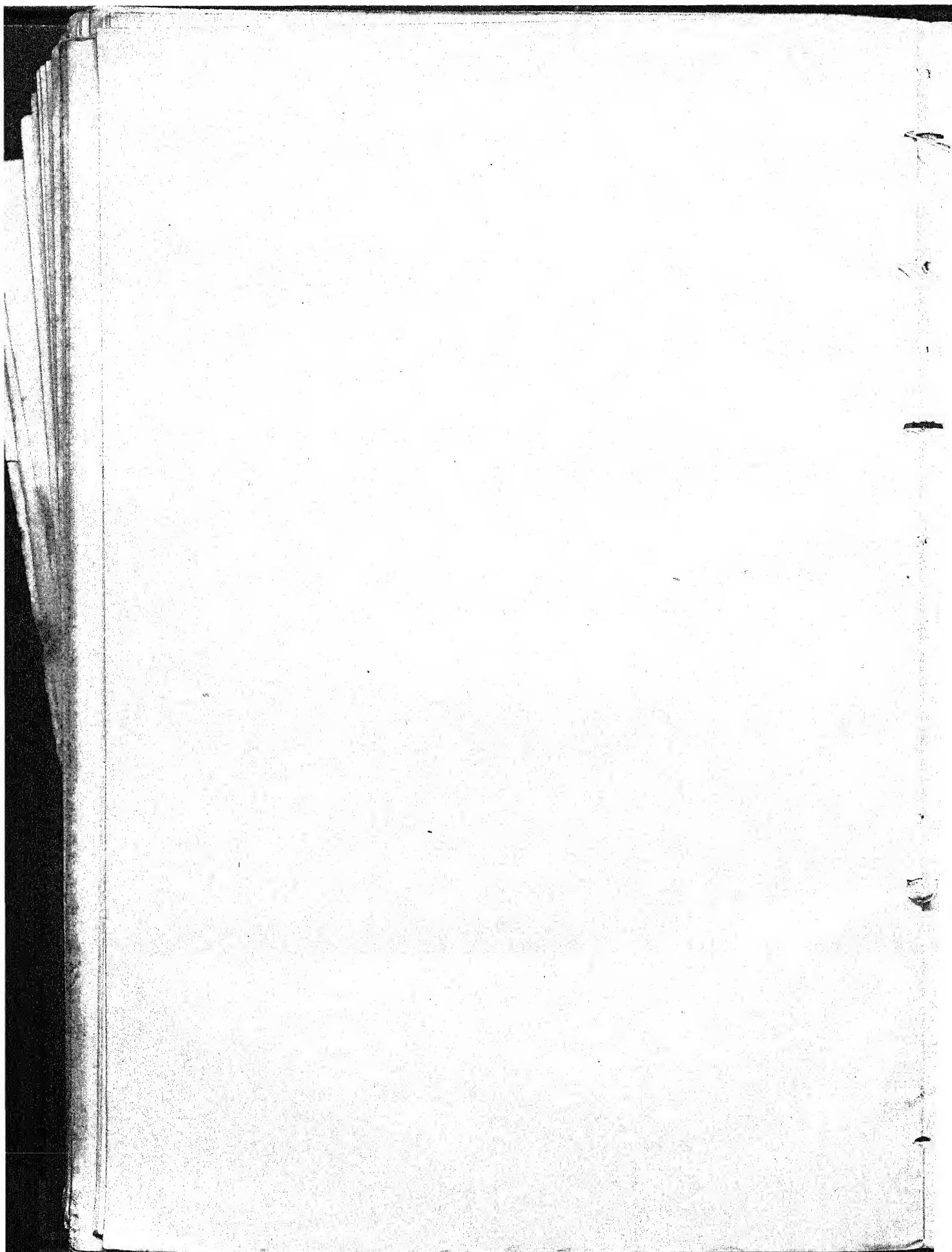
Determinations for total blood serum calcium were made on samples of blood collected daily from 27 cows of the completely milked group and 29 cows of the partially milked group for 5 days prior to the anticipated day of parturition and for 5 days following calving.

Two cows showed mild symptoms and three had parturient paresis in the completely milked group. Eight cows in the partially milked group had

parturient paresis. All cases of parturient paresis occurred in the Jersey breed.

## REFERENCES

- (1) CLARK, E. B., AND COLLIP, J. B. A study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modifications. *J. Biol. Chem.*, 63: 461-464. 1925.
- (2) DRYERRE, H., AND GREIG, R. Further Studies in the Etiology of Milk Fever. Oliver and Boyd, London. 1928.
- (3) FISH, P. A. The Physiology of Milk Fever. III. The Blood Phosphates and Calcium. *Cornell Vet.*, 19: 147-160. 1929.
- (4) GARRISON, E. R., AND TURNER, C. W. The Effect of Udder Irrigation and Milking Interval on Milk Secretion. *Mo. Agr. Expt. Sta. Research Bull.* 234. 1936.
- (5) PETERSEN, W. E., AND RIGOR, T. V. Relation of Pressure to Rate and Quality of Milk Secreted. *Proc. Soc. Exptl. Biol. Med.*, 30: 254-256. 1932.
- (6) SJOLLEMA, B. Über die Bedeutung der Elektrolytkonstellation für den Organismus. Der Biochemie der Gebärpause von Kuhen. *Biochem. Z.*, 200: 300-308. 1928.





## THE VALUE OF HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS, WHEN USED IN UDDER WASHES, IN REDUCING THE PLATE COUNT OF MILK<sup>1,2</sup>

E. M. KESLER, G. H. WATROUS, JR., C. B. KNODT, AND P. S. WILLIAMS

*Department of Dairy Husbandry, The Pennsylvania State College, State College*

Various bactericidal agents now are in general use in solutions for washing the udders of cows prior to milking. Comparatively little information is recorded regarding their value in reducing the plate count of the milk produced. Scales and Kemp (6) reported that 2 minutes was too short a period for chlorine to yield dependable sterility. Other workers, Bryan *et al.* (2) and Waugh *et al.* (9), have shown that no viable organisms were present in recommended concentrations of chlorine. Spurgeon *et al.* (8) compared the bactericidal activity of hypochlorites and quaternary ammonium compounds. When applied to teats which had been inoculated with a suspension of *Streptococcus agalactiae*, these germicidal solutions did not eliminate all the organisms present but did destroy 90 per cent of those that would have remained after ordinary rinsing of teats with non-germicidal solutions. Mueller *et al.* (5) found that approximately 0.3 per cent of cow feces or nonfat milk solids produced a significant decrease in germicidal potency of a 200 p.p.m. quaternary ammonium solution. Keith and Reaves (4) washed udders with quaternary ammonium compounds, chlorine, and plain water. They believe the quaternary ammonium compounds are effective sanitizing agents. Byers and Ewalt (3) reported a reduction of 34.2 per cent in the plate count of milk produced when the udders of cows were washed with chlorine solutions.

Definite information relative to the value of solutions of these substances in washing the udders of dairy cows previous to milking apparently is needed.

### EXPERIMENTAL PROCEDURE

Four groups of five cows, each as similar as possible on the basis of level of milk production, age, and stage of lactation, were selected. All were fed and managed alike. Their udders were washed with clean water preparations as follows: Water alone, 200 p.p.m. chlorine, 400 p.p.m. chlorine, 200 p.p.m. quaternary ammonium compound, and 400 p.p.m. quaternary am-

Received for publication November 5, 1947.

<sup>1</sup> The experimental data in this paper are taken from a thesis presented by E. M. Kesler in partial fulfillment of the requirements for the degree of Master of Science in Dairy Husbandry, Pennsylvania State College.

<sup>2</sup> Authorized for publication October 31, 1947, as paper no. 1402 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

monium compound. A generally used commercial sodium hypochlorite powder was used for preparing the chlorine solution. Similarly, a widely used commercial preparation containing 10 per cent alkyl-dimethyl benzyl-ammonium chlorides of high molecular weight was used as a source of quaternary ammonium compounds. Two gallons of each of these solutions were prepared at a temperature of 125° F. just prior to milking, using amounts of the bactericides in accordance with manufacturers' directions. A clean turkish towel was used for each solution. Washing was done throughout by the same person. The udder and flanks of the cow were scrubbed thoroughly with the towel, which had been removed from the solution and folded wet. The cloth then was rinsed in the solution, wrung dry, and used to wipe the udder. Only one cow was washed with each water preparation. Two streams of milk were removed from each teat into a strip cup. The milking machines were attached 1 minute after the washing was begun.

Previous to each milking time the milking machines were taken apart and scrubbed thoroughly with a detergent. They then were rinsed in clear, warm water, reassembled, and the complete unit autoclaved at 15 lb. pressure for 20 minutes. Before autoclaving, each teat cup was covered with paper foil which was not removed until the machine was to be attached to the udder. Milking was done by the same persons and at the same time each evening. All five cows were milked simultaneously. Each cow was milked dry and machine stripped only.

When the machine was removed from the cow, a sample of the milk was obtained from the pail with a sterile milk thief and placed in a sterile sample bottle. The samples were iced until plated. They were transported to the laboratory and plated on tryptone-glucose-extract-milk agar within 0.5 hour. Plating and counting were done according to Standard Methods for the Examination of Dairy Products (1). This procedure was repeated 5 days with treatments randomized in a Latin square design so that no cow received the same treatment more than once. Use of the five machines was randomized so that the same machine was used only once on each cow and only once with each treatment during the individual trial.

A separate Latin square design and different cows were used for each of the first four trials. This experiment was conducted on only the evening milking. During the last period, it seemed desirable to determine the difference in response, if any, between evening and morning milking. This will be designated as Trial V, which followed the same Latin square design and used the same cows as Trial IV and was run on the mornings of the same days.

#### RESULTS

During the course of Trial I, one cow suffered an attack of acute mastitis. In 2 days, the bacteria per ml. in her milk increased from around 1,000 to

70,000. On two occasions the milker allowed the teat cups to touch the bedding as he was attaching the machine. This resulted in visible sawdust in the milk and exceptionally high plate counts. The results on this trial are shown in table 1. An analysis of variance run on the data showed no significant difference between treatments, possibly due to the small number of observations.

TABLE 1  
*The plate counts of milks obtained in trial I<sup>a</sup>*

Cow no.	Day 1	Day 2	Day 3	Day 4	Day 5
1	Quat. ammonium 200 p.p.m. 760	Chlorine 400 p.p.m. 740	Chlorine 200 p.p.m. 610	Quat. ammonium 400 p.p.m. 1,300	Water 1,700
2	Chlorine 200 p.p.m. 1,100	Quat. ammonium 400 p.p.m. 1,000	Chlorine 400 p.p.m. 9,800 <sup>b</sup>	Water 1,000	Quat. ammonium 200 p.p.m. 70,000 <sup>b</sup>
3	Water 540	Chlorine 200 p.p.m. 740	Quat. ammonium 200 p.p.m. 1,900	Chlorine 400 p.p.m. 1,500	Quat. ammonium 400 p.p.m. 650
4	Chlorine 400 p.p.m. 1,700	Water 1,100	Quat. ammonium 400 p.p.m. 510	Quat. ammonium 200 p.p.m. 1,600	Chlorine 200 p.p.m. 840
5	Quat. ammonium 400 p.p.m. 1,900	Quat. ammonium 200 p.p.m. 2,000	Water 92,000 <sup>c</sup>	Chlorine 200 p.p.m. 60,000 <sup>c</sup>	Chlorine 400 p.p.m. 1,100

<sup>a</sup> Expressed as bacteria per ml.

<sup>b</sup> Mastitis.

<sup>c</sup> Teat cup touched bedding.

Trials II, III, IV and V were run with no further occurrence of contamination. Data for the five trials are shown in table 2. The mean figures shown for Trial I exclude the four instances when the count was high due to uncontrolled influences.

TABLE 2  
*The effect of hypochlorite and quaternary ammonium solutions, when used in udder washes, upon the plate count of milk<sup>a</sup>*

Trial no.	Chlorine 200 p.p.m.	Chlorine 400 p.p.m.	Quaternary ammonium 200 p.p.m.	Quaternary ammonium 400 p.p.m.	Water
I	823 <sup>b</sup>	1,260 <sup>b</sup>	1,565 <sup>b</sup>	1,072	1,085 <sup>b</sup>
II	2,208	2,370	2,812	2,756	1,946
III	1,438	1,372	930	1,448	1,528
IV	2,461	1,109	2,176	1,198	2,174
V	5,821	2,170	2,234	1,180	1,390
Mean .....	2,550	1,656	1,943	1,531	1,625

<sup>a</sup> Mean of 5 observations on each treatment expressed as bacteria per ml.

<sup>b</sup> Mean of 4 observations.



An analysis of variance was run on Trials II, III and IV, which represent the trials on evening milkings with no data missing. No significant difference in treatments could be observed. There was a highly significant difference between cows.

An analysis then was run on all five trials, using the missing data technique (7) for Trial I. Again there was no statistically significant difference between treatments. The variations due to cows were highly significant.

A comparison of the evening and morning counts between Trials IV and V was made. The mean count for the 25 morning observations was 2,559 bacteria per ml.; for the corresponding evening counts the mean was 1,824. This difference was not statistically significant.

#### SUMMARY AND CONCLUSIONS

The effect of various udder washes upon the plate count of milk was studied using two concentrations of chlorine, two concentrations of quaternary ammonium, and clean water. Milking was done with previously autoclaved milking machines and the raw milk plated on tryptone-glucose-extract-milk agar before growth could take place. No differences in count could be attributed to treatments. A large degree of variation was observed between individual cows. On 25 pairs of observations, no significant difference in counts could be observed between night and morning milkings.

#### REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th Ed. New York, N. Y. 1941.
- (2) BRYAN, C. S., DARBY, C. W., MALLMAN, W. L., AND CORBETT, A. C. The Role of Disinfectants in the Control of Mastitis. *J. Milk Technol.*, 5(2): 77. 1942.
- (3) BYERS, J. H., AND EWALT, H. P. The Value of Chlorine in Producing Low Bacterial Count Milk. *J. Dairy Sci.*, 26(3): 277-281. 1943.
- (4) KEITH, R. H., AND REAVES, P. M. The Use of Quaternary Ammonium Compounds for Washing the Udders of Dairy Cows. *J. Dairy Sci.*, 30(8): 564. 1947.
- (5) MUELLER, W. W., SEELEY, D. B., AND LARKIN, E. P. Determining the Germicidal Potency of Quaternary Ammonium Compounds and Their Use in Dairy Sanitation. *J. Dairy Sci.*, 30(8): 519. 1947.
- (6) SCALES, F. M., AND KEMP, M. The Sterilizing Quality of Chlorine Solutions under Different Conditions. *J. Milk Technol.*, 2(5): 215-221. 1939.
- (7) SNEDECOR, G. W. Statistical Methods Applied to Experiments in Agriculture and Biology. The Iowa State College Press, Inc., Ames, Iowa. 1946.
- (8) SPURGEON, K. R., ELLIKER, P. R., HARPER, W. J., AND FROEDGE, J. R. Effectiveness of Hypochlorite and Quaternary Ammonium Germicides in Destroying *Streptococcus agalactiae* in a Mastitis Sanitation Procedure. *J. Dairy Sci.*, 30(8): 519-520. 1947.
- (9) WAUGH, R. K., ELLIKER, P. R., HILTON, J. H., AND BULLARD, J. F. The Chlorine Tolerance of Certain Mastitis Bacteria. *J. Dairy Sci.*, 24(6): 506-507. 1941.

# THE NUTRITION OF THE NEWBORN DAIRY CALF. I. CHANGES IN THE TRYPTOPHAN CONTENT OF THE BLOOD PLASMA FOLLOWING BIRTH AND THE INGESTION OF COLOSTRUM

T. S. SUTTON AND G. C. ESH<sup>1</sup>

*Department of Dairy Husbandry and the Institute of Nutrition and Food Technology,  
The Ohio State University and The Ohio Agricultural Experiment Station*

Recent work at the University of Illinois (6) has shown that calves do not require a dietary source of nicotinic acid when fed a nicotinic acid-free synthetic milk. These calves were placed on a synthetic milk diet after receiving colostrum for 48 hours and were continued on the experimental diet for 12 weeks. A study of the urinary excretion of nicotinic acid and its metabolic products, nicotinamide, nicotinuric acid and N<sup>1</sup>-methylnicotinamide, showed that the total excretion dropped rapidly following colostrum feeding, and then remained at a fairly constant level throughout the remainder of the experimental period.

Contemporary work (7, 9, 10) has provided evidence that tryptophan is the precursor used in the *in vivo* synthesis of nicotinic acid. The relatively high blood plasma nicotinic acid values observed following a 48-hour colostrum-feeding period by the Illinois workers (6), plus the fact that other workers (8) have reported rather low nicotinic acid values for cows' colostrum, suggest the desirability of studying the tryptophan content of colostrum and the changes which occur in plasma concentration following colostrum feeding.

A review of the literature revealed no information on the concentration of tryptophan in colostrum or in newborn calf blood plasma. However, it is known that the proteins of milk are relatively well supplied with this essential amino acid. Data in the literature show 0.31 to 0.32 per cent of tryptophan in whole milk powder (3), from 1.09 to 1.31 per cent of tryptophan in casein (2, 3, 4, 5, 11), and from 1.74 to 2.66 per cent tryptophan in lactalbumin (2, 3, 4, 5). The nature and concentration of proteins in cows' colostrum suggest that it should supply an abundance of tryptophan for the nutrition of the newborn calf.

## EXPERIMENTAL PROCEDURE

Tryptophan determinations were made on the first- and second-milking colostrum from 10 cows and on the blood plasma of 13 calves at the time

Received for publication November 7, 1947.

<sup>1</sup> A graduate student at The Ohio State University supported by the Government of India.

of birth and on the third, seventh, and fourteenth day following birth. Determinations also were made on the blood plasma of 10 of these calves on the twenty-first day following birth. These calves all were born in the Ohio State University Dairy Herd during August and September, 1947. Tryptophan determinations also were made on the whole milk and blood plasma of 15 cows for comparison. Samples of the colostrum as well as of the milk were taken from the complete milking.

The *p*-dimethylaminobenzaldehyde method of Bates (1) as modified by Graham *et al.* (2) was employed for tryptophan estimation. An Evelyn photoelectric colorimeter with a 550-m $\mu$  filter was used. The *K* value was obtained by carrying out the procedure with known quantities of *l*-tryptophan<sup>2</sup> and checked with known quantities of *d-l*-tryptophan.<sup>3</sup>

#### RESULTS

The analytical data obtained are presented in the tables. It will be noted that the tryptophan level in blood plasma is quite low in the newborn calf, averaging 0.46 mg. per g., wet basis (table 1). In every instance there

TABLE 1  
*Changes in the blood plasma tryptophan of calves following birth*

Calf no. <sup>a</sup>	Blood plasma tryptophan				
	Age in days				
	0 <sup>b</sup>	3	7	14	21
	(mg./g.) <sup>c</sup>				
42S	0.46	1.05	1.10	1.04	0.86
41S	0.46	0.99	1.05	0.84	0.75
503H	0.47	1.16	1.16	0.99	0.82
43S	0.43	0.55	0.65	0.62	.....
355A	0.56	1.28	1.01	0.92	0.96
357A	0.75	1.43	1.53	1.43	1.12
329G	0.41	0.71	0.74	0.66	0.64
330G	0.41	0.81	0.84	0.91	0.55
331G	0.45	0.87	0.82	0.70	0.81
332G	0.44	0.95	0.77	0.47	0.89
272J	0.40	0.98	0.86	0.76	0.76
373J	0.48	0.70	0.91	0.76	.....
507H	0.34	0.81	1.14	1.09	.....
Av.	0.46	0.94	0.96	0.86	0.81

<sup>a</sup> The letter following the number designates breed: S = Brown Swiss, H = Holstein, A = Ayrshire, G = Guernsey, J = Jersey.

<sup>b</sup> These samples all were taken before the first feeding of colostrum.

<sup>c</sup> Wet basis.

was a marked increase during the first 3 days, and the average on the third day was approximately double that of the newborn calf. The highest aver-

<sup>2</sup> Supplied through the courtesy of Dr. F. E. Deatherage, Department of Agricultural Chemistry.

<sup>3</sup> Supplied through the courtesy of Merek and Co., Rahway, New Jersey.



age level was observed on the seventh day, following which there was a slight decline. At no time during the first 21 days was a level attained which was as high as that of the adult cow, although in a few instances on the third, seventh, and fourteenth days values were recorded which were within the range found for adult cows, as shown in table 2.

TABLE 2  
*Tryptophan content of blood plasma and milk of dairy cows*

Cow no. <sup>a</sup>	Lactation no.	Days in milk	Blood plasma tryptophan	Milk tryptophan
			(mg./g.) <sup>b</sup>	(mg./g.) <sup>b</sup>
191A	10	44	1.54	0.78
214A	10	146	1.80	0.82
269A	4	35	1.38	0.62
410H	3	75	1.54	0.71
433H	2	218	1.20	0.73
460H	1	13	1.20	0.68
277G	3	28	1.02	0.70
221G	6	250	1.55	0.84
275G	2	16	1.39	0.78
19S	1	69	1.20	0.69
11S	3	236	1.53	0.81
7S	9	39	1.51	0.75
335J	1	17	1.12	0.82
337J	1	48	1.60	0.75
325J	2	48	1.20	0.87
Av.	.....	.....	1.38	0.75

<sup>a</sup> The letter following the number designates breed: A = Ayrshire, H = Holstein, G = Guernsey, S = Brown Swiss, J = Jersey.

<sup>b</sup> Wet basis.

Table 3 shows colostrum to be a rich source of tryptophan. On a wet basis, first-milking colostrum contains about five times as much as normal milk. Second-milking colostrum is about three times as high in tryptophan as normal milk. One pound of average first-milking colostrum would provide approximately 1.74 g. of tryptophan, while a pound of normal milk would supply only about 0.34 g.

The tryptophan content of a 20-lb. lot of fat-free moisture-free colostrum which had been obtained from the first and second milkings of several cows was compared with that of samples of spray-dried and drum-dried commercial nonfat-dry-milk solids. Analyses of these materials gave results of 13.5, 7.9, and 7.7 mg. of tryptophan per g. for the dry colostrum, the spray-dried powder, and the drum-dried powder, respectively. These results are in agreement with what one might expect, since a greater proportion of the nonfat solids of colostrum is protein and a greater proportion of the protein of colostrum consists of fractions which are somewhat higher in tryptophan than casein.

These results suggest that the high excretion rate of nicotinic acid and its metabolic products found by the Illinois workers (6) may have resulted

from the high tryptophan consumption during the first 48 hours. It seems logical to assume that nicotinic acid synthesis in the very young calf must be dependent upon some dietary precursor since microbiological synthesis

TABLE 3  
*Tryptophan content of colostrum*

Cow no. <sup>a</sup>	Tryptophan	
	1st milking	2nd milking
	(mg./g.) <sup>b</sup>	(mg./g.) <sup>b</sup>
373A	4.61	3.15
265A	4.60	2.42
191A	4.62	3.26
269A	5.94	3.93
273H	4.32	3.55
464H	2.82	2.30
460H	4.90	3.16
337J	1.42	1.05
281G	3.15	1.75
277G	2.16	1.20
Av.	3.85	2.57

<sup>a</sup> The letter following the number designates breed: A = Ayrshire, H = Holstein, J = Jersey, G = Guernsey.

<sup>b</sup> Wet basis.

in the rumen is a doubtful source of this nutrient at so early an age. On the other hand, it may be that a relationship exists between tryptophan and nicotinic acid similar to that known to occur between methionine and choline.

A study of the effects of colostrum consumption on the excretion of nicotinic acid and its precursors is being contemplated.

#### SUMMARY

A study of the tryptophan content of the blood plasma of calves showed average values of 0.46, 0.94, 0.96, 0.86, and 0.81 mg. per g. wet basis for the newborn calf and at 3, 7, 14 and 21 days of age, respectively.

The amount of tryptophan in first-milking colostrum was found to be about five times as high and that of the second milking about three times as high as that of normal milk.

These results are discussed in the light of tryptophan being the possible precursor for the *in vivo* synthesis of nicotinic acid.

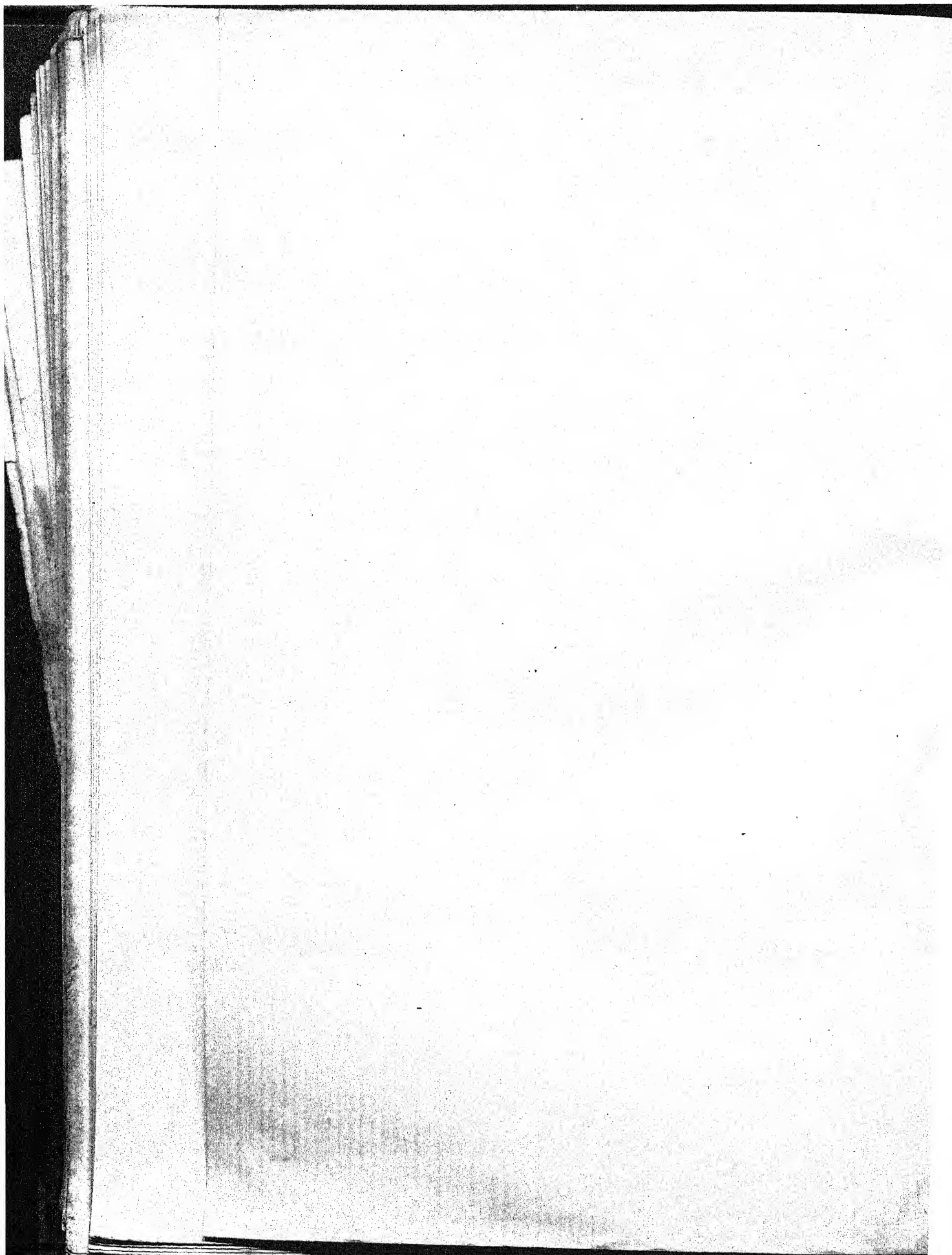
The authors acknowledge with gratitude the assistance of Mr. H. E. Kaeser, who obtained many of the blood samples used in this work.

#### REFERENCES

- (1) BATES, R. W. A Rapid Method for Quantitative Determination of Tryptophane. J. Biol. Chem., 119: vii. 1937.

- (2) GRAHAM, C. E., SMITH, E. P., HIER, S. W., AND KLEIN, D. An Improved Method for the Determination of Tryptophane with p-dimethylaminobenzaldehyde. *J. Biol. Chem.*, 168: 711-716. 1947.
- (3) GREENE, R. D., AND BLACK, A. The Microbiological Assay of Tryptophane in Proteins and Foods. *J. Biol. Chem.*, 155: 1-8. 1944.
- (4) GREENHUNT, I. T., SCHWEIGERT, B. S., AND ELVEHJEM, C. A. Hydrolysis Procedures for the Determination of Tryptophane in Proteins and Foodstuffs by the Microbiological Procedure. *J. Biol. Chem.*, 165: 325-332. 1946.
- (5) HORN, M. H., AND JONES, D. B. A Rapid Colorimetric Method for the Determination of Tryptophane in Proteins and Food. *J. Biol. Chem.*, 157: 153-160. 1945.
- (6) JOHNSON, B. C., WIESE, A. C., MITCHELL, H. H., AND NEVENS, W. B. The Metabolism of Nicotinic Acid and Its Role in the Nutrition of the Calf. *J. Biol. Chem.*, 167: 729-736. 1947.
- (7) LUECKE, R. W., McMILLEN, W. N., THORP, F., AND TULL, CAROLYN. The Supplementary Effect of Tryptophane and Nicotinic Acid in Low Protein Rations for Weaning Pigs. *J. Animal Sci.*, 5: 408. 1946.
- (8) PEARSON, P. B., AND DARNELL, A. L. The Thiamine, Riboflavin, Nicotinic Acid and Pantothenic Acid Content of Colostrum and Milk of the Cow and Ewe. *J. Nutrition*, 31: 51-58. 1946.
- (9) PEARSON, P. B., SCHWEIGERT, B. S., AND WILKINING, M. C. The Apparent Synthesis of Nicotinic Acid from Tryptophane by the Horse. *J. Animal Sci.*, 5: 406. 1946.
- (10) ROSEN, F., HUFF, J. W., AND PEARLZWEIG, W. A. The Effect of Tryptophane on the Synthesis of Nicotinic Acid in the Rat. *J. Biol. Chem.*, 163: 343-344. 1946.
- (11) SULLIVAN, M. X., AND HESS, W. C. A Study of the Various Procedures for the Estimation of Tryptophan. *J. Biol. Chem.*, 155: 441-446. 1944.





## A COMPARISON OF VACUUM AND STEAM DISTILLATION FOR DETERMINING THE VOLATILE ACIDITY OF EVAPORATED MILK

P. G. MILLER, P. L. ZIMMERMAN, AND E. B. OBERG

*Carnation Company Research Laboratories, Milwaukee, Wisconsin*

The acidity and changes in acidity of milk are known to be of importance in milk processing and storage. Many workers have shown that heat causes the formation of acids in milk. As early as 1895 Cazeneuve and Haddon (2) reported that the acid formed by heating was mainly formic acid. This view is in accordance with the recent work of Gould (7) and Gould and Frantz (8). Since most workers report the chief volatile acid produced by heating milk to be formic, the importance of other volatile acids appears questionable.

The use of steam distillation for removing volatile acids from sterilized evaporated milk may or may not be the best procedure. Destruction of milk constituents during steam distillation is indicated by a marked browning of the milk and liberation of volatile sulfides (at proper pH). Although reports show that the steam distillation of raw skim milk or whole milk resulted in the formation of no appreciable amount of formic acid, (7, 8) this does not show what steam distillation may do to heated milk. Also, volatile acids other than formic possibly may be affected. For instance, glycerides of lower fatty acids may be split during the steam distillation of milk.

The use of vacuum distillation in removing volatile constituents from foods has been suggested by Fischbach (5) and has been used successfully by other workers. Vacuum distillation seemed to be a logical method for removing the volatile acids from milk without the use of excessive heating. This investigation was concerned with a comparison of vacuum distillation and steam distillation as a means of studying formic and total volatile acids in heated milk.

### EXPERIMENTAL PROCEDURE

*Vacuum distillation.* The distilling setup consisted of pyrex ground-joint equipment. Essential pieces were a 3-l. round-bottom distillation flask, a 300-mm. spiral (Graham) condenser, a 1-l. receiving flask, and suitable connecting tubes and adapters. Constant pressure was maintained by means of a Cartesian diver-type manostat (6).

In the vacuum method finally adopted 500 ml. of reconstituted milk (1 part evaporated milk to 1.2 parts water) was acidified to pH 1.5 (approximately) by running in 100 ml. of *N* sulfuric acid while the milk

Received for publication November 12, 1947.

was being agitated with a mechanical stirrer. The acidified milk was distilled at a pressure of 24 mm. mercury and at a rate of approximately 400 ml./hr. The boiling temperature was 24–25° C. The volume of the distilland was maintained constant by running boiled distilled water, from a calibrated flask protected by a soda-lime tube, into the distilling flask through a glass tube and stopcock arrangement, and checking the volume of water run in against the volume of distillate.

For the determination of total volatile acids, 600 ml. of distillate was collected and titrated under nitrogen to phenolphthalein with 0.1 *N* sodium hydroxide. A blank correction of 0.09 ml. of 0.1 *N* sodium hydroxide for the amount of alkali required to change the indicator in 600 ml. of boiled distilled water was deducted from the titration. Total volatile acids in the distillate were calculated as formic acid.

For the determination of formic acid the entire distillate then was concentrated to about 100 ml. by boiling with 200 ml. of 1 per cent barium carbonate suspension. The barium carbonate was filtered off and the formic acid determined by the A.O.A.C. gravimetric procedure (1).

*Steam distillation.* The apparatus for steam distillation was essentially the same as that used for vacuum distillation except for the necessary provision for a steam generator and a soda-lime tube connected to the receiving flask outlet in place of vacuum equipment.

The distillation procedure was similar to the vacuum distillation except for the use of steam at atmospheric pressure and the acidification of the diluted evaporated milk sample. The diluted sample was acidified in this case with only 80 ml. of *N* sulfuric acid and 20 ml. of water to lower the pH to approximately 2.0 (as against pH 1.5 and a much-lower temperature with the vacuum method).

The steam distillation procedure differed from that used by Gould and Frantz (8) and Gould *et al.* (9) in the following details: (a) In the present work the size of the sample was doubled. (b) Volatile acids were collected in the distillate rather than in a barium carbonate trap. (c) The ratio of distillate to distilland was 1:1 instead of 3.33:1.

Total volatile acids and formic acid in the steam distillate were determined by the procedure employed with the vacuum distillate.

## RESULTS

*Distillation curves.* Calculation of formic acid and total volatile acids in the milk was based on distillation curves of formic acid determined in the particular apparatus used. The curves presented in figure 1 were plotted from data obtained by distilling dilute aqueous solutions of pure formic, *n*-butyric, and caproic acids under the same conditions and in the same apparatus used in distilling milk samples. The method of plotting on inverted semi-log paper was copied after Dyer (4). Actually, the log of



the percentage of volatile acid remaining in the distilland was plotted against total volume of distillate collected, giving a straight line. However, since the main purpose of the figure is to show the per cent of acid recovered in the distillate, the data were plotted on inverted semi-log paper.

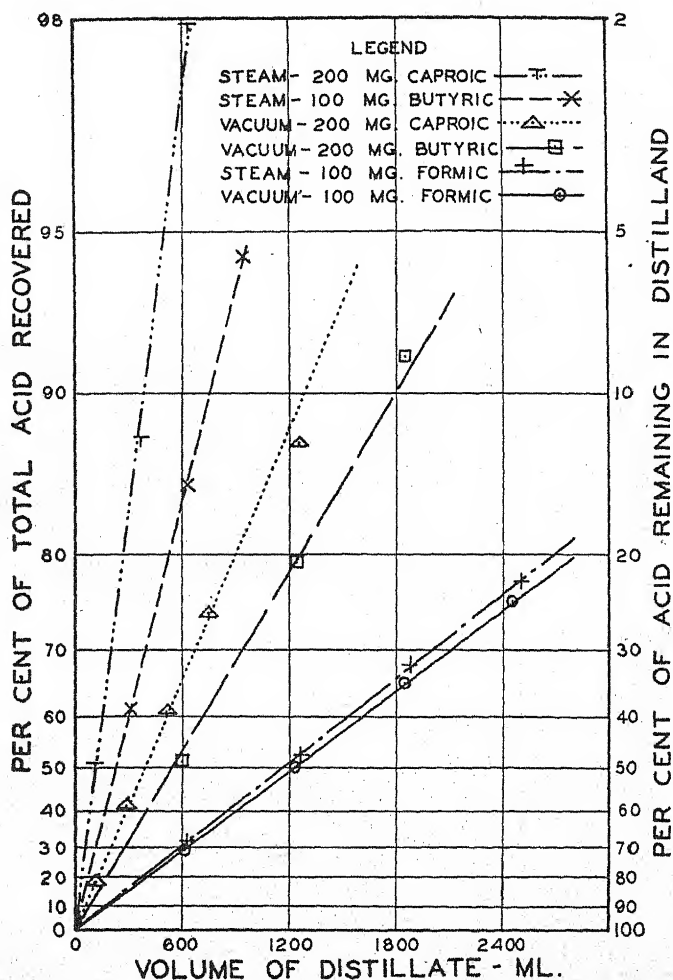


FIG. 1. The rate of vacuum and steam distillation of various acids from aqueous solutions under experimental conditions similar to those used with milk.

The rates of distillation of formic acid with steam and vacuum were about the same, whereas *n*-butyric acid and caproic acid were distilled much more rapidly with steam. Assuming that the rate of distillation of formic acid from the diluted, acidified milk samples was the same as the rate from acidified water, the per cent of the total formic acid in any given volume

of distillate could be determined from the vacuum or steam distillation curve, and thus the amount of formic acid originally present in the sample could be calculated. For example, 600 ml. of distillate (by the vacuum method) would contain 28.7 per cent of the total formic acid in the sample.

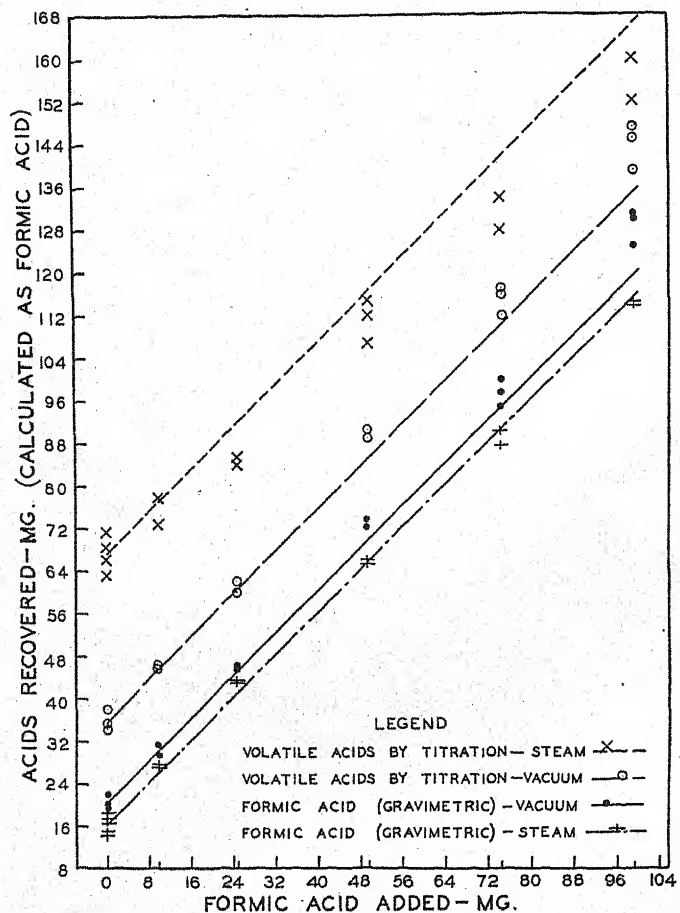


FIG. 2. The recovery of formic acid added to 500-ml. samples of reconstituted sterilized evaporated milk as determined by vacuum and steam distillations.

If the distillate was found to contain 10 mg. of formic acid, the total formic acid in the sample would be 34.8 mg.

In the case of the total volatile acidity determinations, an arbitrary figure was calculated. From the total volatile acidity (as formic acid) in the distillate, the amount in the sample was estimated on the basis of the formic acid distillation curve. This gave values which undoubtedly are high but which permitted a rough comparison of total volatile acidity,

formic acid values, and, by difference, the volatile acids not formic in the distillates.

*Recovery of formic acid added to sterilized evaporated milk.* For recovery experiments, 48 cans of fresh commercial sterilized evaporated milk were placed in a refrigerator until used. Determinations of volatile acidity and formic acid at intervals during the course of the recovery experiments showed no detectable increase in these constituents up to the time the work was completed.

Amounts of solution containing from 10 to 100 mg. of formic acid were added to diluted evaporated milk samples by running the solution through a capillary into milk which was being agitated with a mechanical stirrer. Approximately 600 ml. of distillate from the 600 ml. of diluted acidified sample was collected by vacuum or steam distillation. Titrations of total volatile acids and gravimetric determinations of formic acid were made on the distillates. Values were corrected to 100 per cent recovery on the basis of the vacuum or steam distillation curve for formic acid.

Figure 2 is a graphic representation of data obtained in determinations of total volatile acid and gravimetric formic acid by the steam and vacuum distillation methods. The four lines were drawn through points obtained by taking the sum of the average acid value obtained on the control samples by each method and the amount of formic acid added. Thus, these lines represent values which *should have been obtained* if the recovery of the added formic acid were 100 per cent in all cases. The points plotted show actual values obtained on individual samples containing from 0 to 100 mg. of added formic acid.

The differences in base values obtained on control samples are clearly shown here. Total volatile acidity as determined by the steam distillations was almost double that determined by the vacuum method. The base value for formic acid determined gravimetrically was higher by 3.9 mg. with the vacuum method than with the steam procedure.

With the vacuum distillation procedure, values for total volatile acidity and formic acid in the control group of samples were quite consistent considering the small quantities present in this milk. The results indicate that the method of calculating the amount of formic acid in the milk from the comparatively small amount recovered in the distillate was at least fairly accurate. The recovery of formic acid calculated on the basis of the titration of the distillate agreed well, in most cases, with the figure calculated on the basis of the gravimetric formic acid determination. Recoveries were slightly high, but generally within the range of accuracy to be expected in work of this kind.

With the steam distillation procedure, recoveries of added formic acid by the gravimetric method were very satisfactory in amounts of 50 mg. or more. With smaller amounts of formic acid the recoveries tended to run high. A



point of particular interest here was the erratic and generally low recovery of formic acid as calculated from the titrations of the steam distillate, with recoveries from 58.7 to 105.7 per cent, the average being 83.8 per cent.

The low recovery of formic acid by titration of the steam distillates apparently was due to the fact that values for volatile acids not formic were lower in distillates from samples to which formic acid had been added, since gravimetric determinations of formic acid in these same distillates showed no loss. The vacuum procedure did not give this difference between titration and gravimetric values. As shown in figure 3, there seemed to be a definite relationship between the amount of formic acid added to the milk

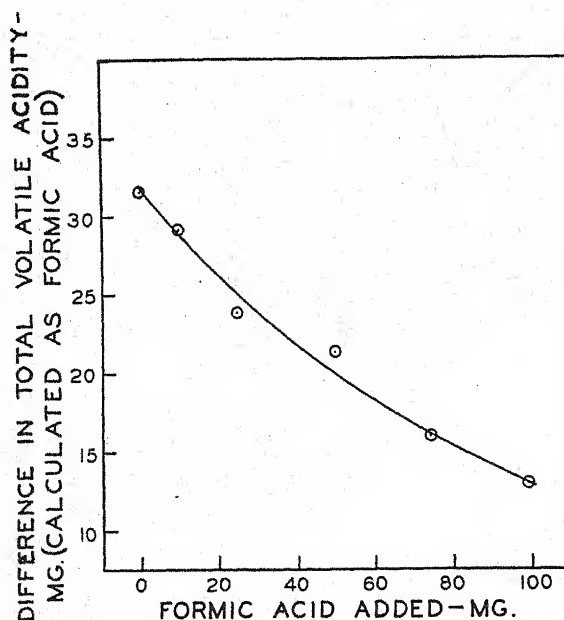


FIG. 3. The effect of amount of added formic acid on the difference between steam and vacuum distillation results for total volatile acidity.

and the *difference* in volatile acidity values between steam and vacuum distilled samples; *i.e.*, as the amount of added formic acid was increased, the difference in volatile acidity between steam and vacuum distilled samples decreased. Consideration of volatile acids not formic may give an explanation for these results.

*Volatile acids not formic.* Calculation of volatile acids not formic was made on steam and vacuum distilled samples by subtracting the gravimetric formic acid values from the total volatile acidity obtained by titration. In figure 4, volatile acids not formic were plotted against added formic acid. This demonstrated that: (a) Values for volatile acids not formic were much

higher by the steam distillation. (b) The steam distillation showed a definite drop in volatile acids not formic when formic acid was added to the milk. (c) The vacuum distillation values for volatile acids not formic remained fairly constant when the formic acid content of the milk was increased.

Possible explanations of the higher volatile acidity values obtained with steam distillation may be: (a) evolution of carbon dioxide during the steam

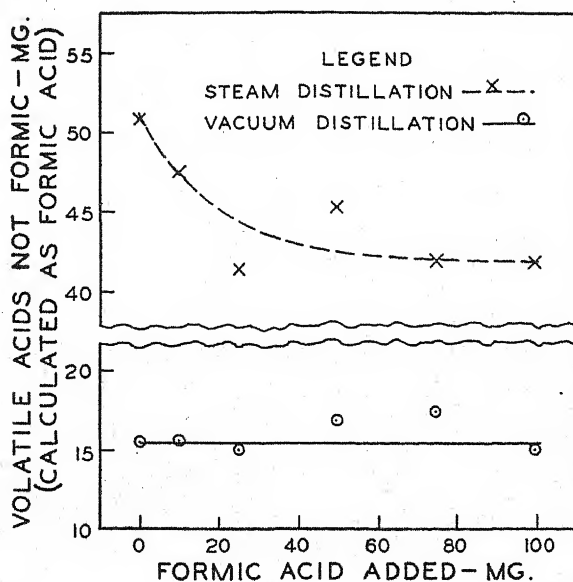


Fig. 4. The relation of amount of added formic acid to the volatile acids not formic as determined by vacuum and steam distillation.

distillation, (b) hydrolysis of glycerides of the lower fatty acids, and (c) more rapid distillation of butyric and higher volatile acids with steam than with vacuum.

The effect of boiling the steam and vacuum distillates to eliminate carbon dioxide is shown in table 1. In these experiments the distillate was titrated with sodium hydroxide under nitrogen as usual. Then standard hydrochloric acid was added in amount equivalent to the sodium hydroxide used in titration. The distillate was heated to boiling as rapidly as possible, boiled for 30 seconds, cooled under protection of a soda-lime tube, and titrated again with sodium hydroxide.

Boiling resulted in considerable loss of acidity in the steam distillate but slight loss of acidity in the vacuum distillate. The amount of formic acid, as determined by the gravimetric procedure, was not decreased appreciably by boiling either steam or vacuum distillate. Negative tests for sulfides on

the steam distillate eliminated the possibility that hydrogen sulfide was causing the high titrations. By placing barium hydroxide solution in the receiving flask during the steam distillation, it was found that carbon dioxide was evolved even after 2 hours of distillation and aspiration with carbon dioxide-free nitrogen. This supports the hypothesis that a part of the difference between volatile acidity values as determined by vacuum and steam distillation was due to dissolved carbon dioxide in the steam distillate. Since a marked browning of the milk occurs during steam distillation, the evolu-

TABLE 1  
*Effect of boiling evaporated milk distillate on total volatile acidity titration*

Distillation procedure	HCOOH added	0.1 N NaOH required for approximately 600 ml. of distillate		
		Unboiled	Boiled	Difference
	(mg.)	(ml.)	(ml.)	(ml.)
Steam	0	4.23	3.22	1.01
	24.8	5.64	4.22	1.42
	49.6	7.63	6.04	1.59
Vacuum	0.0	2.19	2.18	0.01
	9.9	2.86	2.66	0.20
	49.6	5.57	5.44	0.13
	74.4	7.15	6.91	0.24

tion of carbon dioxide during this treatment would be expected. Tarassuk (10) and Coulter (3) have observed carbon dioxide evolution during browning of milk by heat. These reactions are reported to be accelerated by oxygen (10) and inhibited by reducing agents such as formaldehyde and sodium bisulphite (11).

Even after making correction for the loss of acidity on boiling of the steam distillate, its acidity would be higher by about 34 per cent than that of the vacuum distillate on samples to which no formic acid was added, indicating that other factors contributed to the difference in volatile acidity values obtained by the two methods.

Steam distillation of 5-g. of pure tributyrin homogenized with water and distilled under conditions used on milk was tried and resulted in hydrolysis of 3.5 per cent of the tributyrin in 1.5 hours. Vacuum distillation of tributyrin in water did not hydrolyze any of the glyceride. This indicated the possibility of splitting glycerides of lower fatty acids during the steam distillation of milk. However, mixed glycerides such as occur in milk fat probably would be more stable than tributyrin, and so the error due to hydrolysis of glycerides during the steam distillation may or may not be great.

From observations of the distillation curves (Fig. 1) it is evident that the more rapid distillation of butyric and caproic acids with steam could account



for at least part of the difference in volatile acidity as determined by vacuum and steam distillation in these experiments, since total volatile acids in the sample were calculated on the basis of the distillation rate curves of formic acid. However, the amounts of individual acids of higher molecular weight than formic in the distillates were not known, and it is impossible to determine from data obtained just how much this error in calculating total volatile acids contributed to the difference in values obtained by steam and vacuum distillation.

The available data do not show the specific cause for the decrease in volatile acids not formic obtained by steam distillation when formic acid was added to the milk. The limited results obtained by titrations before and after boiling of steam distillates do not indicate that this decrease was due to inhibition of evolution of carbon dioxide by the added formic acid. There seems to be a possibility that formation and/or distillation of part of the volatile acids not formic was inhibited by the presence of formic acid.

#### SUMMARY

1. A method of determining formic acid and volatile acid values in heated milk by means of vacuum distillation at constant volume is presented. Results of experiments to determine the recovery of added formic acid from sterilized evaporated milk by the steam and vacuum distillation procedures are discussed.

2. The formic acid content of reconstituted evaporated milk averaged about 32 mg./l. by steam distillation and about 40 mg./l. by vacuum distillation.

3. The total volatile acid content of reconstituted evaporated milk, expressed as formic acid, averaged 134 mg./l. by steam distillation and 70 mg./l. by vacuum distillation.

4. The vacuum and steam distillation procedures both gave satisfactory recovery of added formic acid from sterilized evaporated milk by the gravimetric method.

5. There was no evidence of production of formic acid during steam distillation as far as the accuracy of the methods could detect.

6. Titrations of volatile acids on the steam distillates were considerably higher than on the vacuum distillates. The difference could be due to continuous evolution of carbon dioxide from the milk during steam distillation, the more rapid distillation rate of butyric and caproic acids with steam, and/or splitting of glycerides of fatty acids during steam distillation.

7. The addition of formic acid to the milk appeared to reduce the amount of volatile acids not formic obtained by steam distillation.

8. The vacuum distillation showed an advantage over steam distillation where a consideration of the total volatile acids in the distillate was desired.

## REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 6th Ed. Washington, D. C. Pp. 535-537. 1945.
- (2) CAZENEUVE, P., AND HADDON, E. Sur les Causes de la Coloration et de la Coagulation du Lait par la Chaleur; Formation d'Acide Formique aux Depens de la Lactose. Lyon Medical, 79(28): 345-348. 1895.
- (3) COULTER, S. T. Our Newer Knowledge Regarding the Manufacture of Powdered Whole Milk. Food Technol., 1(2): 208-215. 1947.
- (4) DYER, D. C. A Method of Steam Distillation for the Determination of the Volatile Fatty Acids, Including a Series of Colorimetric Qualitative Reactions for Their Identification. J. Biol. Chem., 28: 445-473. 1917.
- (5) FISCHBACH, H. Low Pressure, Low Temperature System for Decomposition Studies in Food. J. Assoc. Official Agr. Chemists, 28(3): 639-643. 1945.
- (6) GILMONT, R. Theory and Operation of a Cartesian Diver Type of Manostat. Ind. Eng. Chem., Anal. Ed., 18(10): 633-636. 1946.
- (7) GOULD, I. A. The Formation of Volatile Acids in Milk by High Temperature Heat Treatment. J. Dairy Sci., 28(5): 379-385. 1945.
- (8) GOULD, I. A., AND FRANTZ, R. S. Formic Acid Content of Milk Heated to High Temperatures as Determined by the Steam Distillation Procedure. J. Dairy Sci., 29(1): 27-31. 1946.
- (9) GOULD, I. A., WEAVER, E., AND FRANTZ, R. S. Lactic Acid in Dairy Products. IV. Effect of Storage of Evaporated Milk on Lactic and Formic Acid Production, on pH, and on Acidity and Formol Titrations. J. Dairy Sci., 29(1): 33-40. 1946.
- (10) TARASSUK, N. P. Effect of Oxygen on Color and Flavor of Heated Milk. Food Inds., 19(6): 781-783. 1947.
- (11) WEBB, B. H. Color Development in Lactose Solutions during Heating with Special Reference to Evaporated Milk. J. Dairy Sci., 18: 81-96. 1935.

# CURD TENSION TEST AND CURD NUMBER TEST APPLIED TO MARKET HOMOGENIZED MILK IN PHILADELPHIA— DEFINITION OF A SOFT CURD MILK

BERNHARD SPUR

*The Children's Hospital of Philadelphia, Pennsylvania*

Since the curd number test was described in 1942 (3), this method as well as the curd tension test has been applied regularly in this laboratory to homogenized milks marketed in Philadelphia. The present paper gives a survey of the results and discusses the relationship between curd tension, which deals with the toughness of the curd when milk coagulates, and the curd number, which deals with the size of the curds formed.

The term "soft curd milk" is entirely of American origin and until recently has been practically unknown in other countries. Hill (2) was the first to call attention to the variance in the curd-forming properties of milk, and he designed a special apparatus, manipulated by hand, for testing the toughness of the curd formed under standard conditions. Hill selected a temperature of 95° F. (35° C.) and prescribed a special coagulation solution.

The curd test later was subjected, by a specially appointed committee, to a critical study which resulted in detailed directions for the performance of the test (1). The Hill curd tension meter has been replaced by a mechanically operated apparatus designed by Chambers and now generally used. The curd number test (3) was developed in this laboratory to study closely another aspect of curd formation, namely, the size of the curd. In connection with this study, a baby feeding study was carried out (4).

It generally is agreed that a low curd tension in milk is desirable, especially for infants and people with weakened digestion. A low curd tension is indicative of a soft curd, but even more important is the formation of small curds that will yield a large surface for the action of the digestive juices.

The main objective of this study has been to investigate the relationship between curd tension and the formation of curds as measured by the curd number test, and especially to determine at which curd tension a formation of generally small curds is assured in homogenized milk.

## EXPERIMENTAL

A period of one year, June, 1946, to June, 1947, was selected to include all possible seasonal variations. In all, 208 curd number tests, as well as curd tension tests, were performed on market homogenized milks.

Received for publication November 19, 1947.



The results of all curd tension values over 10 g. and their corresponding curd numbers are recorded in table 1. Curd tension values below 10 g. frequently were found, indicating extremely soft curd milks. These samples were regarded as without significance for this study, in which the upper

TABLE 1  
*The relationship of curd tension to curd number*  
(June, 1946-June, 1947)

No. of samples	Curd tension	Corresponding curd nos.		
		Max.	Min.	Av.
22	10	228	201	214
20	11	229	200	211
18	12	236	200	210
17	13	229	195	210
16	14	231	195	207
23	15	216	195	205
10	16	221	178	202
5	17	214	167	185

limit value for curd tension and the corresponding lower limit value for curd number, characterizing soft curd milks, were sought. Table 1 shows curd tensions from 10 to 17 g. and corresponding curd numbers. Briefly, the curd numbers are arrived at by adding the per centum weight of the curds, divided into three different sizes, large, medium, and small, after having multiplied these figures by one, two, and three, respectively. The presented figures total 131 in number, which means that the rest of the curd tensions, 77 or 37 per cent, were below 10 g. The variance in curd numbers from 10 to 16 g. is not great. None of the figures except one of 178, corresponding to curd tension 16, was below 195. Only five curd tensions of 17 g. were recorded and none over this amount; of these five, the three

TABLE 2  
*The curd numbers of samples with high curd tensions (17-29)*

No. of samples	Curd tension	Corresponding curd nos.		
		Max.	Min.	Av.
8	17	214	135	185
4	20	191	171	178
4	21	180	143	166
3	22	184	164	174
3	23	156	131	139
2	29	171	149	160

relatively low curd numbers, 167, 169 and 175, respectively, indicate the downward trend when the curd tensions increase beyond 16.

From the records taken before 1946, all curd numbers corresponding to a curd tension of 17 g. are recorded in table 2. Of these eight figures, only

two are over 200. The average is the same (185) as for the one-year period tabulated in table 1. Over a period of about 6 years, a few curd tensions of 20 g. and over were encountered. These are tabulated in table 2. Because of their extreme scarcity, they do not have any significance whatsoever in the total picture of curd tensions and curd numbers but are interesting because they show the downward trend in curd numbers with increasing curd tensions. Of special interest are the milks with curd tensions of 20 g., as this is the upper limit value for a milk labeled as "soft curd" according to rules in some states. The average of the four recorded curd tensions of 20 g. is 178, and only one is close to 200. It may seem a little strange that the average curd numbers for curd tensions 22 g. and 29 g. are higher than the average curd numbers for curd tensions 21 g. and 28 g., respectively, but this is only because of the relatively few tests involved. With more tests the higher curd tensions undoubtedly would give corresponding lower average curd numbers. This demonstrates, however, that milks with closely similar curd tensions often vary widely in their curd numbers.

#### DISCUSSION

In the curd number test study (3) the milk curds were divided into three sizes: large curds, caught by a sieve with 0.5-inch mesh; medium-size curds, caught by a sieve with 0.10-inch mesh; and small curds, caught by a sieve with 0.01-inch mesh.

Breast milk usually has curds only of the small size and accordingly will get the highest curd number rating of 300. In the baby feeding study connected with the curd formation study (4), the great majority of milks fed to the babies had curd numbers of 200 and above (1). This milk was well tolerated, and it was suggested at that time that a milk with curd number 200 and over should be labeled as a soft curd milk.

Hundreds of curd specimens have been studied in an effort to determine what sort of curd combinations represent a curd number of about 200 (195-205). Theoretically it is possible to reach this curd number in many different ways. Even a mix of 50 per cent large curds and 50 per cent small curds with no curds of the middle size would give a curd number of 200. Of course, this combination would not be desirable and such a case never has been encountered. Usually a considerable number of the middle-size curds are present, and for a curd number around 200, the large curds will range from 5 to 15 per cent of the total curd weight. The curd numbers of homogenized milks usually are considerably above 200 and consequently show no large curds at all. Furthermore, it is a matter of experience that the large curds for a curd number of 200 are relatively small or close to the mesh size of 0.5-inch in diameter.

Since milk with a curd number of 200 and above, corresponding to a curd tension of 15 g. or less, is well tolerated by infants in general (4),

and since milks with curd tensions above 15 g. contain an increasing number of specimens with curd numbers below 200 as the curd tension increases, it seems reasonable to label milks with curd tensions of 15 g. or below as "soft curd" milks, meaning both "soft" and "small curd" milks.

#### SUMMARY

The curd tensions and corresponding curd numbers (curd sizes) of market homogenized milk in Philadelphia have been recorded over the period of one year. On the basis of this material, combined with experiences from baby feeding studies and more than 5 years of constant testing of curd tensions and curd numbers, it is suggested that a "soft curd" milk be defined as a milk with a curd tension of 15 g. or below. Since this corresponds to a curd number of 200 or above, it indicates a milk with both soft and small curds.

#### REFERENCES

- (1) DOAN, F. J., Chairman. Final Report of Committee on Methods of Determining the Curd Tension of Milk. *J. Dairy Sci.*, 24(9): 825-827. 1941.
- (2) HILL, R. L. The Test for Determining the Character of the Curd from Cow's Milk and Its Application to the Study of Curd Variance as an Index to the Food Value of Milk for Infants. *J. Dairy Sci.*, 6: 509. 1923.
- (3) SPUR, B., AND WOLMAN, I. J. The Curd Number Test. A Method of Testing the Curdling Qualities of Milk. *J. Dairy Sci.*, 25(5): 409-425. 1942.
- (4) WOLMAN, I. J., BOROWSKI, S., NICHOLAS, RAE, AND SPUR, B. Soft Curd Homogenized Milk in Infant Feeding. *J. Pediat.*, 21(1): 45-72. 1942.



## VARIATIONS IN YIELD OF MILK UNDER THE PENKEEPING SYSTEM IN BRAZIL

GERALDO G. CARNEIRO

*Department of Animal Production, Belo Horizonte, Minas Gerais, Brazil*

AND

JAY L. LUSH

*Iowa State College, Ames*

The program for dairy cattle improvement in the state of Minas Gerais in Brazil includes investigating the effects of the special conditions existing under the penkeeping system (*sistema de retiros*) of dairy cattle management. This system is widely used in the *Zona da Mata* (originally a forested region) in the southeastern part of Minas Gerais. The penkeeping system and some of the problems encountered already have been described by Rhoad (5) and by Carneiro (2). Some of the findings from these studies appear to be of general interest.

In the penkeeping system the cattle are kept on pasture the year around. They are divided into *retiros* or pens of 20 to 40 head each. The cows are with their calves in the pasture during the day but are penned up in the evening and remain separated from the calves until they are milked in the morning. As a rule the only feed they get is pasture, but in recent years some use has been made of chopped sugar cane, grass silage, and even cottonseed meal and wheat bran during the dry season. The climate is hot and the annual rainfall is high, with two definite seasons, a rainy one from October to March and a dry season from April to September. The most important among the pests and diseases include ticks, "*berne*" (*Dermatobia hominis*), flies, worms, foot-and-mouth disease, anthrax, blackleg, pneumonia, and tick fever.

The principal objectives of the present study were to determine: (a) How much the average milk production changed from one year to another and the extent to which these year-to-year changes were part of a general time trend or were only irregular variations. (b) How the milk production varied at different times of the year. (c) The shape of the lactation curve with advancing lactation. The findings concerning the sex ratio and preliminary observations on heritability also are reported.

### EXPERIMENTAL PROCEDURE

The records came from "Niagara" farm near Leopoldina, Brazil. This is a private farm which kept grade Simmenthaler cattle under management

Received for publication November 26, 1947.

<sup>1</sup> Journal Paper No. J-1501 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 31.

practices thought to be typical of the penkeeping system in that region. The records were for lactations begun in the years 1930 to 1937. The number of cows in milk ranged from 233 to 318. Milk yields were measured on the first and the sixteenth of each month. All the data on yield are in liters. Under the penkeeping system either the death of the calf or sickness of the cow affects production sharply. Lactations were considered abnormal and were omitted from this analysis if the cow was seriously sick, as from foot-and-mouth disease, or if the calf died or the cow was sold early in the lactation. Of the 2,177 lactations recorded, only 1,318 (slightly over 60 per cent) were considered normal. Table 1 shows for each year the number of lactations and the means and standard deviations for length of lactation and for milk yield in the normal lactations. The ratio of normal to abnormal lactations fluctuated widely from year to year, as might be expected from the fact that outbreaks of foot-and-mouth or other disease often would cause a heavy loss in some *retiros* or in some years but not in others.

TABLE 1  
*Normal, abnormal and total lactations by years*

Year	No. of lactations			Milk yield (l.)		Lactation period (days)	
	Normal	Abnormal	Total	Mean	$\sigma$	Mean	$\sigma$
1930	194	70	264	1,105	386	289	66
1931	97	136	233	1,144	402	316	82
1932	161	81	242	1,282	344	313	67
1933	75	195	270	1,129	348	287	72
1934	196	56	252	1,207	365	305	64
1935	213	70	283	1,261	413	301	65
1936	187	123	315	1,226	407	285	63
1937	195	123	318	1,242	413	304	61
Summary	1,318	859	2,177	1,209 $\pm 11$	393	299 $\pm 2$	67

#### RESULTS

*Year-to-year differences in yield and in length of lactation period.* Table 1 shows how the means varied from one year to the next. Table 2 shows the analysis of variance between and within years. Since the effect of year on length of lactation only bordered on statistical significance and was slight in any case, its analysis was carried no further.

The mean yield differed from year to year with unmistakable statistical significance. The analysis to test whether those year-to-year differences were wholly irregular or whether a part of them could be attributed to a straight-line trend also is shown in table 2. The trend is an average increase of 16.7 l. per year, ( $Y = 1147.4 + 16.7X$ ) but the yearly means changed too irregularly for statistical significance to be assured. Other

things besides the steady trend (if that actually is real) obviously have much to do with causing the mean to be high in some years and low in others. A rough computation of the variance components indicates that about 40 per cent of the variance caused by year-to-year changes in the mean can be at-

TABLE 2  
*Variation in milk yield between and within years*

Source of variation	d/f	Milk yield		Length of lactation	
		Mean square	F	Mean square	F
Total .....	1,317	154,284	.....	4,423	.....
Regression .....	1	1,924,583	4.16	} 11,099	2.53*
Yearly means from the regression .....	6	462,486	3.05**		
Between cows within years .....	1,310	151,521	.....		
				4,388	.....

\* = Significant.

\*\* = Highly significant.

tributed to the straight-line trend. The other 60 per cent of the year-to-year variance comes from causes which had irregular incidence from one year to the next. However, the year-to-year variations were not a large part of the total causes of individual variation, since the variance component for year-to-year differences, including the trend, is only about 2 per cent of the variance between records made within the same year. The individual variation found between records made within the same year (necessarily by different cows) is so large that even an indicated increase of nearly 1.4 per cent per year is by comparison a small source of variation. This small increase, however, eventually would mean much to the dairy industry if it is real and if it continues for many years.

*Season of year.* Only a portion of the data was used for measuring how yield varied with the season of the year, 50 of the normal lactations being

TABLE 3  
*Variance of daily milk yields between and within months of the year*

Source of variation	d/f	Mean square	F
Total .....	3,876	2.722	.....
Between months .....	11	29.236	11.05**
Within months .....	3,865	2.646	.....

\*\* = Highly significant.

selected from each of the 8 years. The selections were random except that some effort was made to get the calving dates equally distributed among the 12 months, so that stage of lactation would not be confounded with season of year. This was not wholly achieved. The 400 records included slightly more than a fair share which began from March to May and too few which



began in December and January. The 400 lactations provided 3,877 daily milk yields, using only the yields measured on the sixteenth of each month. Table 3 shows the analysis of variance between and within months.

The effect of month is unmistakably significant statistically but is not large enough to be of much practical importance, since the mean square is reduced only about 2.8 per cent by "holding month constant". The present results agree fairly well with those of Rhoad (5), as shown in table 4. The

TABLE 4  
*Mean daily milk yield by months*

Month	Present study		Rhoad's study	
	Mean	Deviation of monthly mean from annual mean	Mean	Deviation of monthly mean from annual mean
	(L.)	(%)	(L.)	(%)
Jan. ....	4.31	+ 6.2	4.42	+10.0
Feb. ....	4.41	+ 8.6	4.57	+11.2
March ....	4.40	+ 8.4	4.42	+ 9.9
April ....	4.33	+ 6.6	4.23	+ 5.2
May ....	4.24	+ 4.4	3.84	- 4.5
June ....	3.96	- 2.5	3.63	- 9.7
July ....	3.77	- 7.1	3.62	-10.0
Aug. ....	3.60	-11.3	3.38	-15.9
Sept. ....	3.60	-11.3	3.62	- 9.9
Oct. ....	3.89	- 4.2	3.95	- 1.7
Nov. ....	4.14	+ 2.0	4.21	+ 4.7
Dec. ....	4.24	+ 4.4	4.42	+ 9.9
General mean .....	4.06	.....	4.02	.....

effect of month is a bit more extreme in Rhoad's data, but the season of year when the yields are below average (after the dry season is well begun) is almost the same. Likewise, the maximum in both sets of data occurs from the middle to near the end of the rainy season. As one evidence that Rhoad's data show more extreme seasonal variations than the present data, the rate of decline from the highest month to the lowest by Brody's (1)

formula,  $k = \frac{\ln Y_2 - \ln Y_1}{t_2 - t_1}$ , yields values of 0.050 and 0.034 per months for

Rhoad's data and for the present data, respectively. The difference can be attributed plausibly to the fact that the cows studied by Rhoad received no supplementary feed, while those used in the present study had some grass silage and some chopped sugar cane during the winter (dry) season. Also, it is possible that the few cases of twice-a-day milking in the present data were more frequent in the dry months, although that is not known for certain.

It seems worth emphasizing that the dry season in the tropics and sub-tropics appears to affect both milk production and growth, primarily through

its effect on the feed supply. This observation checks both with the present study and Rhoad's study and also with the results reported by Schutte (6) on the growth of beef cattle in South Africa. Schutte points out that rainfall rather than *season per se* causes the seasonal deviations, since the maximum growth of feed occurs approximately 3 months after the time of greatest precipitation.

*Shape of the lactation curve.* It is well known that under conditions of dairy management usual in the temperate zone, the lactation curve rises at the beginning of the lactation, reaching a maximum around 30 to 50 days after parturition, and then declines to the end of the lactation period. Whether this relation is the same under the penkeeping system was investigated by sorting the daily yields according to their order in the lactation. Yields measured in the first 14 days after parturition were called "first measurements", those from the 15th to the 29th day were called "second measurements", those from the 30th to the 44th day were "third measurements", and so on. This method of sorting placed all records with the same order of measurement in nearly the same segment of the lactation curve. Necessities of management, such as changing a cow from one *retiro* to another and accidents, of course resulted in failure to obtain some figures for

TABLE 5  
Mean daily milk yields, in liters, at 15-day intervals from calving to the 30th measurement (450 days)

Order of measurement	No. of lactations	Mean yield	Order of measurement	No. of lactations	Mean yield	Order of measurement	No. of lactations	Mean yield
		(l.)			(l.)			(l.)
1	523	4.35	11	1316	4.18	21	579	3.01
2	1113	4.76	12	1313	4.12	22	482	2.95
3	1286	5.00	13	1308	4.04	23	379	2.85
4	1309	4.98	14	1290	3.94	24	288	2.75
5	1314	4.85	15	1264	3.85	25	288	2.75
6	1314	4.64	16	1201	3.67	26	174	2.63
7	1314	4.58	17	1125	3.51	27	136	2.65
8	1315	4.44	18	996	3.35	28	102	2.59
9	1315	4.37	19	836	3.24	29	75	2.58
10	1315	4.32	20	700	3.12	30	59	2.55

daily yields, especially those for yields at the first measurement date after parturition. The average number of days from calving to the first yield recorded was  $18.6 \pm 0.3$ . Many of the cows began to go dry about the time of the 16th measurement (*i.e.*, about 240 days), but a few continued through the 30th measurement. These averages are shown in table 5 and in figure 1.

Table 6 shows an analysis of variance within and between order of measurements. The effect of stage of lactation is statistically significant beyond all doubt and is large enough to be economically important, as somewhat more than 15 per cent of the variance between individual daily yields disappears when stage of lactation is held constant. The correlation be-

TABLE 6  
*Analysis of variance of daily milk yield between and within stages of lactation*

Source of variation	<i>d/f</i>	Mean square	<i>F</i>
Total .....	25,968	2.74	.....
Between orders of measurement	29	38.21	16.5**
Within orders of measurement ..	25,939	2.32	.....

\*\* = Highly significant.

tween yield and order of measurement was  $-0.198$ . That this value was no larger numerically when the reduction in variance was over 15 per cent, of itself shows that the relation was not entirely linear. The straight regression lines shown in figure 1 were fitted separately. The rising one was fitted to the first three measurements and the declining one to the third to 30th measurements. The slope of the rising line is an average increase of 0.314 l. per 15-day interval, while the slope of the declining one is an average decrease of 0.107 l. per 15-day interval. Admittedly the sharp break in the curve at the third measurement is an artifact, resulting from the arbitrary division of the data at this point. The true curve probably sweeps

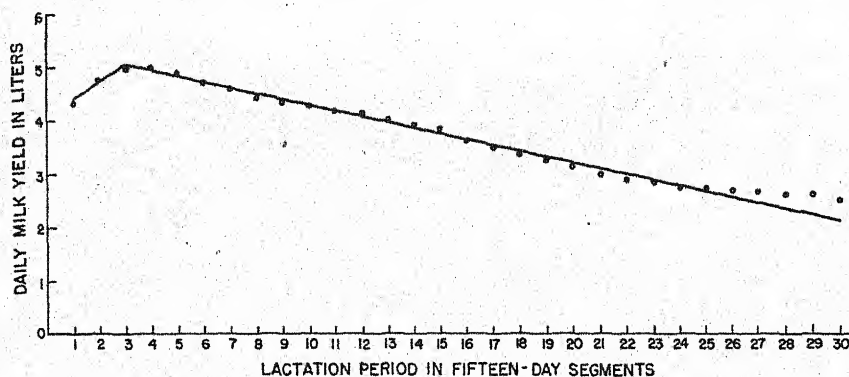


FIG. 1. Yields in successive stages of the lactation period.

smoothly with decreasing slope up toward a maximum and then slowly turns down again. However, as the choice of type of curve had to be arbitrary, further refinements to fit a single curve to the whole set of data did not seem worth while.

A straight line fits most of the declining part of the curve well until near the very end. There the numbers are few and it seems likely that the apparent lessening of the slope may be the result of selection, whereby those



which went dry and dropped out at each interval would mostly have been those which were producing the least at the preceding measurement. The rate of decline in the declining segment amounts to only 2.3 per cent each 15 days at the beginning of that portion, but is as much as 4.1 per cent of the current production by the time the lactation is about 12 months along. If the rate of decline from measurements 3-5 to measurements 24-26 is computed according to Brody's (1) formula (which, however, would make the regression a bit curvilinear), the decline is 2.86 per cent for each 15 days or about 5.7 per cent per month.

These rates of decline are not very different from those reported for dairy data from the type of management usual in temperate regions, although the means are much lower. Thus Brody (1) reports declines per month of 5.3, 5.5, 5.6, and 5.7 per cent, respectively, for groups of Holstein, farrow Guernsey, Jersey and Guernsey cows. It is somewhat surprising that the rates of decline should be so nearly the same in these data from a breed which is more nearly dual purpose, with cows milked only once a day, fed little but pasture, some of which was not very good, and suckled by their calves during the day. Possibly the management holds the production so much below the cow's inherent ability that this level of production remains more stable than it would under better management. However, this is only a tentative suggestion needing more investigation. Perhaps Brody's estimate that scrub cows decline almost 17 per cent per month needs testing on a wider variety of data. The progressive elimination of those which had been producing the least in each preceding month could explain the ap-

TABLE 7  
*Sex ratio among the calves*

Year	Males	Females	Total
1930	149	116	265
1931	108	124	232
1932	130	113	243
1933	135	124	259
1934	129	106	235
1935	154	138	292
1936	173	154	327
1937	164	146	310
Total .....	1,142	1,021	2,163

parent slowness of the decline here after about the 15th measurement, but it could not explain the slowness of the earlier decline. There is no indication that such elimination is a noteworthy factor until about the 26th measurement.

*Sex ratio.* Although not a primary object of this study, the sex distribution by years was tabulated and is shown in table 7. Like most other reports on the sex ratio in cattle, this study shows a slight but statistically significant excess of males. The males comprised 52.8 per cent of the calves.

This compares with 51.8 reported by Crew (3), or the 50.5 by Gowen and Pearl, 49.4 by Roberts, 51.5 by Johansson, 52.2 by Ward, and 49.9 by Engeler, as quoted by Lush (4). The males were in excess every year except 1931 and in that year the difference was small. The statistical test for homogeneity from year to year yields a chi-square value of 5.42, which actually is a bit less than expected for seven degrees of freedom. Therefore, the conclusion is reached that the males were genuinely in excess of the females at Leopoldina, for reasons not known to the authors. This is in agreement with most other studies of the sex ratio in cattle. Whatever factor made the sex ratio depart from exact equality seems to have prevailed over all the years.

*Heritability.* Only a preliminary study of the heritability of individual differences in milk production has yet been made. The still unverified estimates are in the neighborhood of 0.5, which seems rather high as compared with most other studies. However, the herd contained cows of several different kinds of breeding. Some kinds contained distinctly higher percentages of dairy blood than others. This genetic heterogeneity would tend to make the heritability of differences within such a population higher than within a group which were all purebreds or high grades of the same breed. Also, the peculiarities of the penkeeping management, such as whether a cow would let down her milk freely to the hand milker in the morning when accustomed to being milked by the calf during the day, might make a noticeable difference. It is conceivable that the differences in behavior between the cows in this respect could be large and strongly hereditary. Speculation on this point seems unjustified until the existing evidence can be verified and examined from every point of view. The preliminary examination indicates a rather high level of heritability of individual differences in milk production under the penkeeping system.

#### SUMMARY

Records from a large farm in the *Zona da Mata* in Brazil were studied to learn about conditions which affect milk production under the penkeeping system.

Production varied significantly from year to year. Part of this is ascribed to an upward trend with time, but the statistical significance of that trend is not wholly assured. Some of the yearly means deviated rather widely from that trend.

Season of year had significant but rather small effects on the daily yield. The higher yields were toward the middle and end of the rainy season, while the lower ones were late in the dry season.

Production reached a maximum some time around 40 to 50 days after calving. Thereafter it declined in almost a straight line.

The sex ratio showed a slight but statistically significant excess of males. The year-to-year deviations of the sex ratio from the general mean were not statistically significant.

Heritability of individual differences in milk yield under these conditions seems moderately high.

#### REFERENCES

- (1) BRODY, S. Growth and Development. Mo. Agr. Expt. Sta. Research Bull. 105. Pp. 21-26, 50-51. 1927.
- (2) CARNEIRO, G. G. Factors Affecting the Milk Production of Simmenthaler Grade Cows under the Penkeeping System. Iowa State Coll. J. Sci., 13: 249-268. 1939.
- (3) CREW, F. A. E. Animal Genetics. Oliver and Boyd, Edinburgh, Scotland. 420 pp. 1925.
- (4) LUSH, J. L. Animal Breeding Plans. 3rd Ed. The Collegiate Press, Ames, Iowa. 443 pp. (See especially p. 406.) 1945.
- (5) RHOAD, A. O. Production of Brazilian Dairy Cattle under the Penkeeping System. Tierzücht u. Züchtungsbiol. (Reihe B, Z. Zücht.), 33: 105-118. 1935.
- (6) SCHUTTE, D. J. Factors Affecting the Growth of Range Cattle in Semi-arid Regions. Onderstepoort J. Vet. Sci. Animal Ind., 5: 535-617. 1935.





# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

APRIL, 1948

NUMBER 4

## A COMPARATIVE STUDY OF THE BIOCHEMICAL ACTIVITY OF *STREPTOCOCCUS LACTIS*, *STREPTOCOCCUS CITROVORUS*, AND *STREPTOCOCCUS PARACITROVORUS* WHEN GROWN IN COW'S MILK AND SOYBEAN MILK

CHARLES W. GEHRKE<sup>1</sup> AND HARRY H. WEISER

*Department of Bacteriology, The Ohio State University, Columbus*

The importance of acetylmethylcarbinol and diacetyl from the standpoint of imparting a desirable flavor and aroma to butter and other food products is widely known. The value of selected cultures of bacteria for the development of these compounds has been well established through comparative studies on butter made with and without the use of butter cultures. Hammer and Babel (6) state in their review of butter cultures that previous to 1919 it was commonly believed that butter cultures were pure cultures of lactic acid streptococci, although there had been various suggestions that the desirable flavor of butter made from ripened cream was not produced by the lactic acid bacteria growing in the cream. In that year three laboratories established the basis for an understanding of the bacteriology of butter cultures by reporting almost simultaneously that such cultures normally include two distinct types of bacteria. Boekhout and Ott de Vries (1) isolated from sour milk and cream an organism which produced the characteristic and desirable butter culture aroma when grown with an organism of the *Streptococcus lactis* type; Hammer and Bailey (7) found that butter cultures contained organisms, associated with *S. lactis*, which commonly did not curdle milk but which in combination with *S. lactis* gave high volatile acidities; and Storch (14) considered two types of organisms necessary in butter cultures, a lactic acid type and a flavor type. The latter did not coagulate milk or form much acid but produced more volatile acid than the former. The two distinct types of organisms present in the butter cultures commonly used are *S. lactis* or *Streptococcus cremoris*, which primarily attacks the lactose and forms relatively large amounts of lactic acid together with small amounts of secondary products, and *Streptococcus citrovorus* and/or *Streptococcus*

Received for publication September 5, 1947.

<sup>1</sup> Professor of Chemistry, Missouri Valley College, Marshall, Mo.

*paracitrovorus*, which are characterized mainly by the fermentation of citric acid to diacetyl and related compounds, some of which add greatly to the flavor and aroma of butter cultures, certain types of butter, cultured buttermilk, various cheeses and other food products. The numbers of bacteria in active butter cultures, as determined by plate counts, commonly are in the hundreds of millions and may be over 1 billion per ml. In the cultures studied by Hammer (4), *S. lactis* often made up 90 per cent of the flora and only occasionally fell under 75 per cent; in certain cases the flavor type made up only 1 to 3 per cent of the flora.

Price *et al.* (9) found that the lactic acid organisms usually produce from 0.7 to 1.0 per cent acid in milk, with the maximum about 1.2 per cent; most of the acid is lactic. Suzuki *et al.* (15) stated that the group of bacteria represented by *S. lactis* produces from 90 to 98 per cent of the theoretic yield of lactic acid from the sugar fermented, the remainder of the sugar going to alcohols, aldehydes and esters. With 50 cultures of lactic acid streptococci, Sherman and Albus (13) found the acid produced in milk (10 days at 35° C.) ranged from 0.60 to 0.95 per cent, the average being 0.80 per cent.

The high volatile acidities of butter cultures are evident from the odor and are readily detected by chemical procedures. Hammer and Bailey (7) found that the volatile acidities of butter cultures ranged from 31.2 to 37.6 (ml. of 0.1 *N* NaOH to neutralize 1 l. steam distillate from 250 g. culture); the total acidities varied from 0.87 to 1.08 per cent. Cordes and Hammer (3) noted that the volatile acidity of a butter culture increased as the total acidity increased until, in general, it reached 10 to 15 per cent of the total acidity. With 183 butter cultures grown in pasteurized milk, Templeton and Sommer (16) found the volatile acidities averaged 15.43 per cent of the total acidities; with 28 cultures grown in pasteurized milk plus 0.2 per cent citric acid, the value was 23.10 per cent. Boekhout and Ott de Vries (2) found that symbiosis of the two types of butter culture organisms yielded not only flavor but also considerable volatile fatty acid which was acetic.

The recognition by Van Niel *et al.* (17) that acetylmethylcarbinol (AMC) and diacetyl (AC<sub>2</sub>) either are responsible for the aroma of butter or are the principal components of the aroma material soon led to studies on the production of AMC and AC<sub>2</sub> in butter cultures. Schmalfuss (10), by the sense of smell, detected AC<sub>2</sub> in a milk culture of a rod-shaped lactic acid organism and, through analyses, confirmed the identification of this compound. Van Niel *et al.* (17) noted that certain strains of propionic acid bacteria on a special medium (yeast-dextrose-chalk-agar) produced an odor similar to that of a high quality butter. A wide variation in the production of this odor was noted among the strains of propionic acid



bacteria, and a study of the products formed indicated that AMC was related to the typical butter aroma. It also was observed that a dilute aqueous solution of AMC or  $AC_2$  had an odor characteristic of butter. The authors concluded that  $AC_2$  either is responsible for the aroma of butter or is the principal component of the aroma material. Michaelian *et al.* (8) determined the amounts of AMC +  $AC_2$  in satisfactory cultures and also in cultures lacking flavor. The results showed that considerable AMC plus  $AC_2$  was present in satisfactory cultures. The authors also found that cultures contained only small amounts of AMC +  $AC_2$  during the early stages of ripening, while conspicuous increases occurred later. It was observed that early in the ripening pronounced changes in titratable acid or pH had little effect on the amount of AMC +  $AC_2$  present, but later striking increases occurred with little or no change in acidity. Hammer (5) and Michaelian *et al.* (8) made an extensive study of the relationship of AMC and  $AC_2$  to butter cultures. Hammer and Babel (6) compiled an extensive review on the bacteriology of butter cultures. Schmalfluss and Barthmeyer (11, 12) studied the presence of AMC and  $AC_2$  in various food materials and noted that the foods examined contained much more AMC than  $AC_2$ .

Much research has been done on the study of flavor and aroma compounds in milk, butter, foods and other materials. However, to the authors' knowledge, no information has been published on the production of these compounds in soybean milk and its products. This investigation was undertaken to determine the comparative biochemical activity of the butter culture organisms, *S. lactis*, *S. citrovorus*, and *S. paracitrovorus*, in cow's milk and soybean milk.

Soybean or vegetable milk is used extensively throughout Japan and China for infant feeding as well as a food for adults. The introduction of soybean milk to the American people has occurred only recently. Soybean milk has been manufactured in the form of a powder. It has been used with good results in breads and cakes, in creaming vegetables, in custards, in chocolate or cocoa, and in several other food products as a substitute for cow's milk, especially in those countries that find it cheaper to use a vegetable milk. The high nutritive value of soybean milk and its many potential uses indicate that this product will continue to rise in importance as an item in the human diet. The development by butter culture organisms of  $AC_2$  and related compounds in vegetable milk and products made from it may be desirable from a commercial standpoint.

#### EXPERIMENTAL PROCEDURE

The butter cultures used in this investigation were obtained from the Department of Dairy Industry, Iowa State College, Ames. The cultures

were carried in sterile skimmed milk, transferred daily, and incubated at 21° C. until coagulation occurred. The cultures were removed immediately after coagulation and held in the refrigerator at a temperature of approximately 10° C.

Seven-hundred-milliliter samples of skimmed cow's milk and of soybean milk were placed in quart milk bottles, plugged with rubber stoppers, covered with wrapping paper, and sterilized by heating to 100° C. for 20 minutes on 3 consecutive days. The soybean milk used was obtained from Harry Miller, Director of the International Nutrition Laboratory, Mt. Vernon, Ohio. The sterilized samples of milk were inoculated with butter culture organisms (3 ml.) and held at 21° C. for 0, 12, 28, 48, 72, 96, 168 and 216 hours. The hydrogen-ion concentration, titratable acidity, volatile acidity, and  $\text{AMC} + \text{AC}_2$  were determined in duplicate on each sample at the end of each incubation period.

The pH determinations were made on 10-ml. samples of each culture of fermented milk, using a Coleman 3C glass electrode potentiometer.

The titratable acidity was determined by the titration of 10 ml. of the culture with 0.1 *N* sodium hydroxide, using phenolphthalein as the indicator. The end-point taken was that point at which a faint pink color remained for 1 minute. The acidity obtained was expressed as per cent lactic acid.

The volatile acidity was determined by the method of Michaelian *et al.* (8). Two hundred and fifty grams of the cultured milk with 250 ml. of distilled water was steam distilled after the addition of 15 ml. of *N* sulfuric acid. The first 1,000 ml. of distillate was titrated, using 0.1 *N* sodium hydroxide and phenolphthalein. The results were expressed as the ml. of 0.1 *N* sodium hydroxide required to neutralize the acidity. In determining the amounts of flavor and aroma compounds by the procedure of Michaelian *et al.* (8), a 200-g. portion of the milk was distilled with steam after adding 40 ml. of ferric chloride solution to oxidize the  $\text{AMC}$  to  $\text{AC}_2$ . Hydroxylamine hydrochloride, sodium acetate, and nickel chloride solutions were added to the distillate as a mixture. The material was allowed to stand at least 1 day in order to permit complete crystallization; the nickel salt then was filtered into a weighed crucible. The salt was washed with distilled water, dried to constant weight at 110° C., and the results were expressed as the milligrams of nickel dimethylglyoximate per 200 g. of cultured milk.

#### RESULTS AND DISCUSSION

The values obtained for the pH and volatile acidity were comparable in most instances for both types of milk held at the various incubation periods. After cultures of fermented soybean milk were held for 96 to 216 hours, the volatile acidities ranged from 24.2 to 32.3 (ml. of 0.1 *N* NaOH to neutralize 1 l. of steam distillate from 250 g. of culture);

TABLE 1

*The relationship between certain biochemical activities of butter culture organisms when propagated in cow's and soybean milk*  
(Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

	Hours incubated								
	0	4	12	24	48	72	96	168	216
Cow's milk									
pH .....	6.6	6.1	5.7	4.3	4.3	4.3	4.4	4.4	4.3
Titratable acidity <sup>a</sup> .....	0.15	0.20	0.25	0.83	1.12	1.07	1.04	1.08	1.03
Volatile acidity <sup>b</sup> .....	4.1	4.0	4.1	9.6	20.4	23.5	23.4	33.8	27.5
Mg. Ni salt .....	None	None	Trace	10.2	18.6	15.3	18.7	18.2	16.9
Soybean milk									
pH .....	6.1	5.7	5.4	4.6	4.5	4.5	4.5	4.5	4.4
Titratable acidity <sup>a</sup> .....	0.17	0.17	0.24	0.45	0.49	0.51	0.55	0.49	0.51
Volatile acidity <sup>b</sup> .....	3.8	6.4	7.1	10.5	12.5	10.9	24.2	25.0	32.3
Mg. Ni salt .....	None	None	None	None	4.6	9.3	12.5	33.6	21.4

<sup>a</sup> As per cent lactic acid per 10 g. sample.

<sup>b</sup> Values expressed in ml. of 0.1 N sodium hydroxide per 1,000 ml. of distillate.

<sup>c</sup> Milligrams of nickel dimethylglyoximate per 200 g. of culture.



whereas, for cow's milk the values were 23.4 to 27.5 ml. (table 1). The value for the volatile acidity in cow's milk plus 0.15 per cent citric acid averaged 32.6 ml. for an incubation period of 96 to 216 hours; the corresponding value for cultured soybean milk was 33.5 ml. (table 2).

The production of AMC plus  $AC_2$  in soybean milk by butter culture organisms was not evident until after an incubation period of 48 hours; however, upon incubation of cultured soybean milk for 168 to 216 hours, larger amounts of the flavor and aroma compounds were produced in soybean milk as compared to cow's milk (table 1). When the samples were held for 168 hours at 21° C., 33.6 mg. of Ni salt were obtained from 200 g. of cultured soybean milk. In cow's milk held under similar conditions, 18.2 mg. of Ni salt were obtained. In cow's milk a trace of AMC plus  $AC_2$  was formed after a 12-hour incubation period. The results obtained for the production of AMC plus  $AC_2$  in cow's milk are in agreement with those of Michaelian *et al.* (8), who found that satisfactory cultures yielded 10 mg. or more nickel dimethylglyoximate per 200 g., the maximum being 39.5 mg. The results obtained for the cultured soybean milk are comparable.

When the butter culture organisms were grown in cow's milk plus 0.15 per cent citric acid, the AMC plus  $AC_2$  content was found to the extent of 14.2 mg., as the Ni salt, per 200 g. of culture after an incubation period of 12 hours (table 2). The AMC plus  $AC_2$  content increased to 44.7 mg. upon incubation of the cultures for 216 hours. For cultured soybean milk plus 0.15 per cent citric acid the production of AMC plus  $AC_2$  was retarded; 2.4 mg. of the Ni salt were obtained after an incubation period of 24 hours, which increased to 42.3 mg. after 216 hours of incubation. AMC plus  $AC_2$  was not developed as rapidly in soybean milk as in cow's milk, but upon prolonged incubation the results obtained were in close agreement.

The AMC plus  $AC_2$  content of samples of cultured cow's milk and soybean milk held for 72, 96, 168, and 216 hours at 21° C. averaged 17.2 and 19.2 mg. of the Ni salt, respectively (table 1). When 0.15 per cent citric acid was added to the cultures, the average values obtained were: cow's milk, 33.2 mg.; soybean milk, 32.5 mg. (table 2). This represents a 93 and 70 per cent increase in the AMC plus  $AC_2$  content of the cultured cow's milk and soybean milk, respectively.

The values obtained for the titratable acidity in cow's milk and cow's milk to which 0.15 per cent citric acid was added were nearly twice as great as those secured for the cultured soybean milk. Early in the ripening pronounced changes in titratable acid or pH had little effect on the amount of AMC plus  $AC_2$  present, but later significant increases occurred with little or no change in acidity. These results are in agreement with those of Michaelian *et al.* (8).

TABLE 2  
The relationship between certain biochemical activities of butter culture organisms when propagated in cow's and soybean milk modified by the addition of citric acid

(Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

	Hours incubated								
	0	4	12	24	48	72	96	168	216
Cow's milk (0.15 per cent citric acid added)									
pH .....	6.6	6.2	4.8	4.8	4.2	4.0	4.0	4.4	4.4
Titratable acidity <sup>a</sup> .....	0.15	0.21	0.90	0.90	1.04	1.08	1.13	1.15	1.10
Volatile acidity <sup>b</sup> .....	4.3	4.4	10.8	16.1	33.6	27.5	33.5	31.0	33.5
Mg. Ni salt <sup>c</sup> .....	None	None	14.2	36.4	32.0	30.3	28.6	29.5	44.7
Soybean milk (0.15 per cent citric acid added)									
pH .....	6.1	5.6	4.8	4.8	4.3	4.2	4.0	4.5	4.5
Titratable acidity <sup>a</sup> .....	0.16	0.15	0.51	0.45	0.64	0.66	0.68	0.64	0.67
Volatile acidity <sup>b</sup> .....	4.0	6.6	10.8	9.1	20.2	21.2	31.3	31.5	37.7
Mg. Ni salt <sup>c</sup> .....	None	None	Trace	2.4	12.6	36.4	20.9	30.4	42.3

<sup>a</sup> As per cent lactic acid per 10 g. sample.

<sup>b</sup> Values expressed in ml. of 0.1 N sodium hydroxide per 1,000 ml. of distillate.

<sup>c</sup> Milligrams of nickel dimethylglyoximate per 200 g. of culture.

Further data are presented in table 3 for samples of cultured soybean milk to which 0.10, 0.15, 0.20, and 0.30 per cent citric acid was added.

TABLE 3

*The relationship between certain biochemical activities of butter culture organisms when propagated in cow's milk and soybean milk re-enforced with citric acid*

(Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

Hours held at 21° C.	Added citric acid (%)	pH	Titratable acidity <sup>a</sup>	Volatile acidity <sup>b</sup>	mg. of Ni salt <sup>c</sup>
Soybean milk					
0	0.10	6.1	0.17	3.8	None
48	0.10	4.6	0.68	11.5	11.9
72	0.10	4.6	0.62	23.0	23.4
120	0.10	4.3	0.64	33.5	40.1
0	0.20	6.1	0.17	3.8	None
48	0.20	4.6	0.67	12.3	15.0
72	0.20	4.7	0.70	27.0	47.0
120	0.20	4.4	0.77	40.0	44.0
72	0.15	4.4	0.65	21.1	20.3
120	0.30	4.4	0.66	40.2	35.4
Cow's milk					
72	0.15	4.3	1.02	39.3	49.1
120	0.30	4.3	1.04	40.7	46.0

<sup>a</sup> As per cent lactic acid per 10 g. sample.

<sup>b</sup> Values expressed in ml. of 0.1 N sodium hydroxide per 1,000 ml. of distillate.

<sup>c</sup> Milligrams of nickel dimethylglyoximate per 200 g. of culture.

#### SUMMARY AND CONCLUSIONS

A comparative study was made of pH, titratable acidity, volatile acidity, and acetylmethylcarbinol plus diacetyl on samples of cow's milk and soybean milk inoculated with the butter culture organisms, *Streptococcus lactis*, *Streptococcus citrovorus* and *Streptococcus paracitrovorus*.

The values secured for the pH and volatile acidity were comparable in most instances for both types of milk held at the various incubation periods. The value for the volatile acidity in cultured cow's milk averaged 28.3 (ml. of 0.1 N NaOH to neutralize 1 l. of steam distillate from 250 g. of culture) for an incubation period of 96 to 216 hours; the corresponding value for cultured soybean milk was 27.2 ml. For cultured cow's milk and soybean milk plus 0.15 per cent citric acid, the values were 32.6 and 33.5 ml., respectively.

During the early stages of fermentation of cultured cow's milk and



soybean milk, only small amounts of acetylmethylcarbinol plus diacetyl were present, while after 96 hours of incubation at 21° C. appreciable increases in these substances occurred.

Samples of cultured soybean milk held 168 to 216 hours contained larger amounts of acetylmethylcarbinol plus diacetyl than cultured cow's milk; 33.6 mg. of Ni salt were obtained from 200 g. of cultured soybean milk held 168 hours at 21° C.; whereas, in cow's milk held under similar conditions, 18.2 mg. were found.

The acetylmethylcarbinol plus diacetyl content of eight samples of cultured cow's milk and eight samples of soybean milk held 72 to 216 hours at 21° C. averaged 17.2 and 19.2 mg. of the Ni salt, respectively. When 0.15 per cent citric acid was added to the cultures, the values obtained were: cow's milk, 33.2 mg.; soybean milk, 32.5 mg. The addition of citric acid resulted in a 93 and 70 per cent increase in the production by butter culture organisms of acetylmethylcarbinol plus diacetyl in cow's milk and soybean milk.

Acetylmethylcarbinol plus diacetyl was not developed as rapidly in soybean milk as in cow's milk, but upon extended holding of the cultures the results obtained were comparable.

Cultures of cow's milk held at 21° C. for 72 to 216 hours had an average lactic acid content of 1.05 per cent; whereas, cultures of soybean milk held under similar conditions had a lactic acid content of 0.51 per cent. Samples of fermented cow's milk plus 0.15 per cent citric acid held for 72 hours or longer contained nearly twice as much lactic acid as samples of fermented soybean milk.

Early in the ripening pronounced changes in titratable acid had little effect on the development of acetylmethylcarbinol plus diacetyl in cow's milk and soybean milk, but later in the incubation period significant increases occurred with little or no change in acidity.

#### REFERENCES

- (1) BOEKHOUT, F. W. G., AND OTT DE-VRIES, J. J. Aromabildner bei der Rahmsäuerung. Zentr. Bakt., Parasitenk, II, 49: 373-382. 1919.
- (2) BOEKHOUT, F. W. G., AND OTT DE-VRIES, J. J. Aromavormers, bij de roomzuring. Verslag. Landb. Onderzoek. Rijkslandbouwprofsta., 24: 1-12. 1920.
- (3) CORDES, W. A., AND HAMMER, B. W. The Relation between the Volatile and Total Acidity in Starters and in *S. lacticus*. Iowa Agr. Expt. Sta. Res. Bull. 66. 1921.
- (4) HAMMER, B. W. Volatile Acid Production of *S. lacticus* and the Organisms Associated with It in Starters. Iowa Agr. Expt. Sta. Bull. 63. 1920.
- (5) HAMMER, B. W. Some of the Causes for Unsatisfactory Results with Cultured Buttermilk Especially from the Standpoint of Flavor. Proc. Intern. Assoc. Milk Dealers. 24th Ann. Conv., Lab. Section, 21-30. 1931.
- (6) HAMMER, B. W., AND BABEL, F. J. Bacteriology of Butter Cultures: A Review. J. Dairy Sci., 26: 83-168. 1943.

- (7) HAMMER, B. W., AND BAILEY, D. E. The Volatile Acid Production of Starters and of Organisms Isolated from Them. Iowa Agr. Expt. Sta. Res. Bull. 55. 1919.
- (8) MICHAELIAN, M. D., FARMER, R. S., AND HAMMER, B. W. The Relationship of Acetylmethylcarbinol and Diacetyl to Butter Cultures. Iowa Agr. Expt. Sta. Res. Bull. 155. 1933.
- (9) PRICE, F. E., WILSTER, G. H., AND HURD, C. G. Design of Equipment and Method of Preparing Starter for Oregon Creameries and Cheese Factories. Oreg. Agr. Expt. Sta. Bull. 301. 1932.
- (10) SCHMALFUSS, H. Diacetyl ein Stoffwechselprodukt. Z. angew. Chem. 41: 847. 1928.
- (11) SCHMALFUSS, H., AND BARTHMEYER, HELENE. Diacetyl als Aromabestandteil von Lebens und Genussmitteln. Biochem. Z., 216: 330-335. 1929.
- (12) SCHMALFUSS, H., AND BARTHMEYER, HELENE. Nachweis von Diacetyl und Methyl-Acetyl-Carbinol in Lebensmitteln. Z. Untersuch. Der Lebensmitl., 63: 283-288. 1932.
- (13) SHERMAN, J. M., AND ALBUS, W. R. Some Characters Which Differentiate the Lactic Acid Streptococcus from Streptococci of the Pyogenes Type Occurring in Milk. J. Bact., 3: 153-174. 1918.
- (14) STORCH, V. 102 de Beretning fra Forsogslaboratoriet. Udgivet af den Kgl Veterinaer-og Landbohojskoles Laboratorium for Landokonomiske Forsog. Copenhagen. 1919.
- (15) SUZUKI, S. K., HASTINGS, E. G., AND HART, E. G. The Production of Volatile Fatty Acids and Esters in Cheddar Cheese and Their Relation to the Development of Flavor. J. Biol. Chem., 7: 431-458. 1910.
- (16) TEMPLETON, H. L., AND SOMMER, H. H. Use of Citric Acid and Sodium Citrate in Starter Cultures. J. Dairy Sci., 12: 21-36. 1929.
- (17) NIEL, C. B. VAN, KLUYVER, A. J., AND DERX, H. G. Uber das Butteraroma. Biochem. Z., 210: 234-251. 1929.

# THE ROLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS.

## III. MILK LIPIDS, INCLUDING PHOSPHOLIPIDS<sup>1</sup>

G. A. RICHARDSON<sup>2</sup> AND M. S. EL-RAFEY<sup>3</sup>

*Division of Dairy Industry, College of Agriculture, University of California, Davis*

The role of the milk lipids in the foaming of milk is not clearly defined. Van Dam (27), by decreasing the fat content of separated milk from 0.22 to 0.08 per cent, and Mohr and Brockmann (14), by increasing it from 0.04 to 1.0 per cent, concluded that the foaming capacity and foam stability varied inversely with the fat content. Sommer and Horrall (24) found addition of fat to a skim milk-gelatin-sugar mix greatly decreased whipping ability. El-Rafey and Richardson (5) attributed the minimum foaming of skim milk, whey, lactalbumin sols, and blood serum at approximately 27° C. to the presence of fat globules.

Leete (11), on the other hand, from studies with skim milk, milk, and cream, concluded no definite statement could be made regarding the effect of milk fat on foaming without considering temperature. Sanmann and Ruehe (22), failing to find a definite relationship between the fat or solids content of milk and its foaming ability, suggested that the foaming ability of milk from individual cows largely is dependent upon factors which are characteristic of the cow. When these latter factors were controlled; they found that increasing the fat content usually decreased the foaming ability; the reverse was true with respect to solids-not-fat. According to Holm (7), increases in the fat content of milk over that normally present result in increased foaming and great foam stability.

The effect of the physical state of the fat on its influence on foaming has been recognized. Mohr and Brockmann (14) found that milk exhibits greater foaming properties at temperatures at which the fat is liquid than where it is solidified. This appears to be contrary to the theory proposed by Leviton and Leighton (12) that the destructive action of milk fat and other lipids on foam depends upon their ability to spread on water. It has been reported that the concentration of fat in sodium caseinate solutions is of less significance on their foaming capacity than the chemical and physical condition of the fat (19).

Preliminary studies of the role of milk fat in the foaming of milks and

Received for publication October 31, 1947.

<sup>1</sup> This paper represents a portion of the thesis presented by M. S. El-Rafey in partial fulfillment of the requirement for the degree of Doctor of Philosophy, University of California, May, 1941.

<sup>2</sup> Present address, Oregon State College, Corvallis.

<sup>3</sup> Present address, Fouad First University, Gizah, Egypt.



creams suggested not only that the concentration, the chemical composition, and the physical state of the fat must be considered, but also that the nature of the material comprising the interface between the fat globules and the aqueous medium is of even greater significance.

#### EXPERIMENTAL PROCEDURE

The method of measuring foaming capacity and foam stability has been described in a previous publication (4). The milk fat used in this study was obtained by churning fresh cream from mixed herd milk, melting the butter at 50° C., and filtering the decanted fat at the same temperature. The fat was stored at 5° C.

Preliminary investigations showed that soybean phospholipids gave essentially the same results when used as emulsifiers as milk phospholipids isolated from separator slime. Soybean phospholipids,<sup>4</sup> which are a mixture including lecithin, cephalin, and probably inositol phosphatide, were used in these studies.

The natural emulsions were prepared by diluting cream with the appropriate volumes of its separated milk. The artificial emulsions were prepared by dispersing the fat or oil, with or without added phospholipid, in pasteurized separated milk, or other medium, using a two-cylinder hand emulsifier.<sup>5</sup> When stabilizers, such as gum arabic, were used, the fat first was triturated with the powdered gum and distilled water, and the resulting cream, after being diluted to the desired concentration, was passed through the emulsifier.

The size of the fat globules in the creams was determined microscopically using the technic of Cole and Smith (3). Emulsification was considered satisfactory when the globules ranged in diameter from 1 to 11  $\mu$ , with an average diameter of 3.5  $\mu$ . To attain this it sometimes was necessary to pass the mixtures through the emulsifier three to five times.

Phosphorus in the fats was determined colorimetrically (31), the fat being ashed according to the method of Horrall (8).

#### RESULTS

*Effect of fat percentage on the foaming of milk and cream.* At certain temperatures, at least, skim milk and cream yield their own distinctive type of foam, as illustrated in figure 1. In a series of milks and creams of increasing fat contents, both types of foam will be expected to be present at a certain fat content, and somewhere in the series a reversal of predominating types will take place.

<sup>4</sup> "Margo", 70 per cent phospholipid in soybean oil, courtesy of Dr. J. Eichberg, American Lecithin Company, Long Island City, N. Y. "Best Grade Lecithin", courtesy of Mr. D. C. Ingraham, Durkee Famous Food Company, Berkeley, California.

<sup>5</sup> Club Aluminum Products Co., Chicago, Illinois.

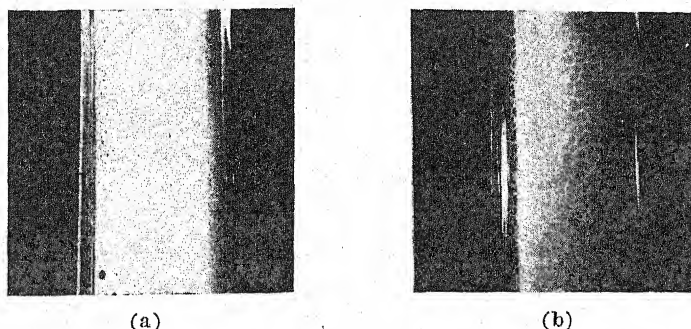


Fig. 1. Types of foam from milk and cream. (a) Skim milk or protein type. (b) Cream or lipoprotein type.

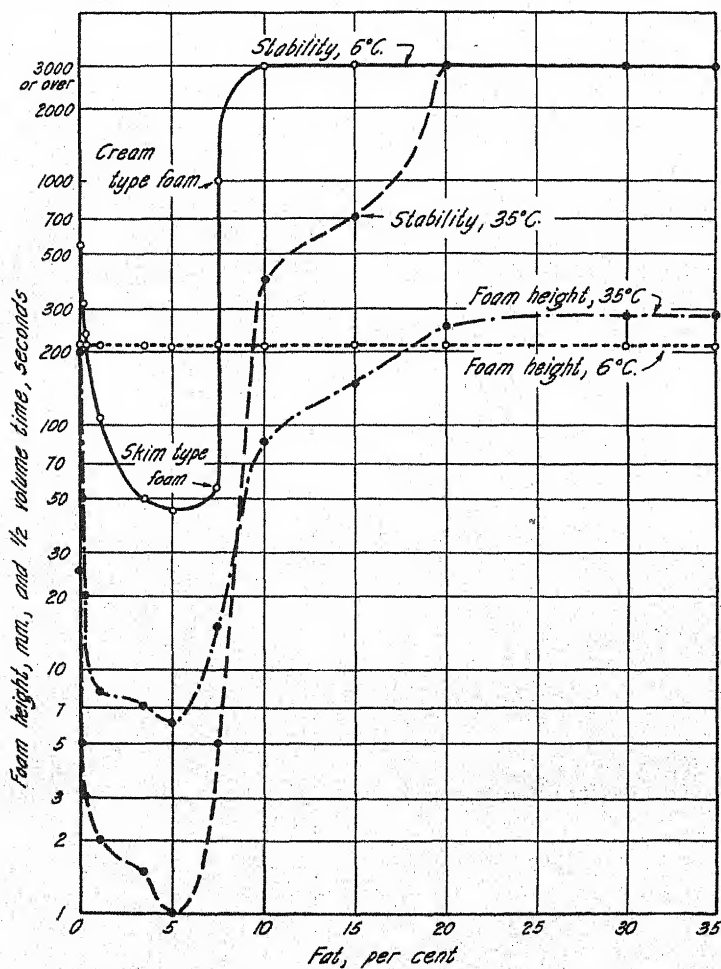


Fig. 2. The effect of increasing the fat content of skim milk from 0.01 to 35.0% on its foaming properties at 6° C. and at 35° C.

A series of samples ranging in fat contents from 0.01 to 35.0 per cent was prepared using raw separated milk and raw cream. As shown in figure 2, at both 6 and 35° C., as the fat content increases, the foam stability first decreases to a minimum value, and this is followed by a rapid increase in stability. With fat concentrations up to between 5.0 and 7.5 per cent, the foam consisted of small, compact cells with a short half-volume time. Above 7.5 per cent of fat, a cream-type or lipoprotein-type of foam predominated. This latter consisted of large cells with distorted lamellae that maintain an increasingly stable structure with increasing fat percentage.

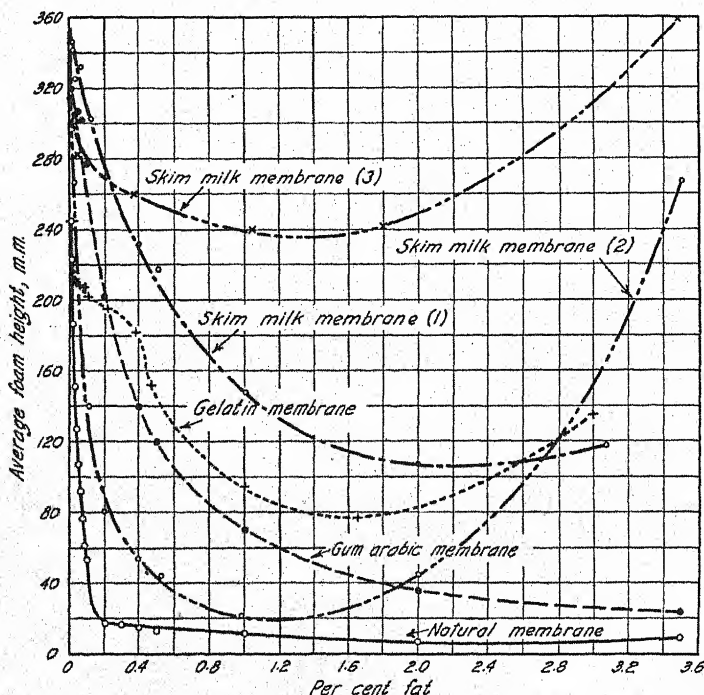


FIG. 3. The effect of the type of stabilizer on the foaming of milk fat emulsions at 35° C. (All emulsions were made by diluting creams, stabilized as indicated, with skim milk. The emulsions for curve (2) differ from those of (1) in that the fat for (2) contained 4% phospholipids added prior to emulsification. Curve (3) represents emulsions of soybean oil containing 4% added phospholipids.)

At 6° C. both types of foam were visible in the 7.5 per cent milk. At 6° C. the foam volume remained constant regardless of the fat content.

*Effect of the emulsifying agent on foaming.* Various emulsions were prepared as follows: (a) Natural raw cream was diluted with raw separated milk to give a series of milks ranging in fat contents up to 3.5 per cent. (b) Artificial emulsions were made by emulsifying milk fat into raw separated milk, gelatin sols, or gum arabic and diluting the resulting



creams with separated milk to give a series of milks of fat contents similar to those in (a). (c) Artificial emulsions were made by emulsifying milk fat or soybean oil, each containing 4 per cent added soybean phospholipids, into raw separated milk and diluting these with the separated milk to obtain the series of milks of varying fat contents. The foaming characteristics of these milks at 35° C. were studied.

The results are shown in figure 3. With the emulsions made with the natural cream, a rapidly progressive decrease in foaming occurs with increasing fat content up to 0.2 per cent fat, after which no marked further decreases occur. The emulsions of milk fat containing the added phospholipids showed a marked resemblance to the natural emulsions up to a fat content of 1 per cent. With increasing fat contents, however, the foaming capacity (and foam stability) increased. The type of this latter foam was distinctive. It was coarse in structure, consisting mostly of five- and six-membered rings. It exhibited a marked glistening and iridescent appearance. The lamellae became very thin and the whole structure collapsed in an explosive manner. This type of foam will be referred to as the "phospholipid" foam. As later experiments will indicate, the 4 per cent added phospholipid is excessive.

The effect of increasing fat contents is not so marked in those emulsions stabilized with skim milk or gelatin. No changes in the nature of the foams were observed with increasing fat contents. With the gum arabic-stabilized emulsions, increasing concentrations of fat exerted a slow but progressive depressing action on foaming. The depressing action of the soybean oil containing added phospholipids was found to be less marked than that of milk fat, but, in the higher concentrations, the typical phospholipid foam was observed.

*Effect of increasing phospholipid content on the foaming of separated milk.* A 2 per cent soybean phospholipid emulsion, prepared by dispersing it in pasteurized skim milk at 50° C. and passing it five times through the emulsifier, was diluted with the skim milk to give a series ranging from 0.0 to 2.0 per cent added phospholipid. Foaming tests were made at 21.5° C. The results are shown in figure 4. As the added phospholipid increases to 0.05 per cent, the foaming tendency decreases. This decrease is about six times that obtained by adding soybean oil in amounts equivalent to that associated with the added phospholipid.

As the concentration of phospholipid increases beyond 0.05 per cent, a typical phospholipid foam begins to appear, first as a coarse unstable foam, similar to that of buttermilk, and followed by foams of increasing height, compactness, iridescence and stability.

*Effect of phospholipid concentration in the fat on foaming of milk emulsions.* Four 10 per cent fat emulsions were prepared by emulsifying,

in pasteurized skim milk, milk fat containing 0.0, 0.45, 0.7, and 1.0 per cent added soybean phospholipid. The foaming properties were studied at various temperatures between 5 and 55° C. Figure 5 shows that each emulsion yielded foams of minimum stability at temperatures between 27 and 35° C. As the phospholipid content increased, the protein-type foam, characteristic

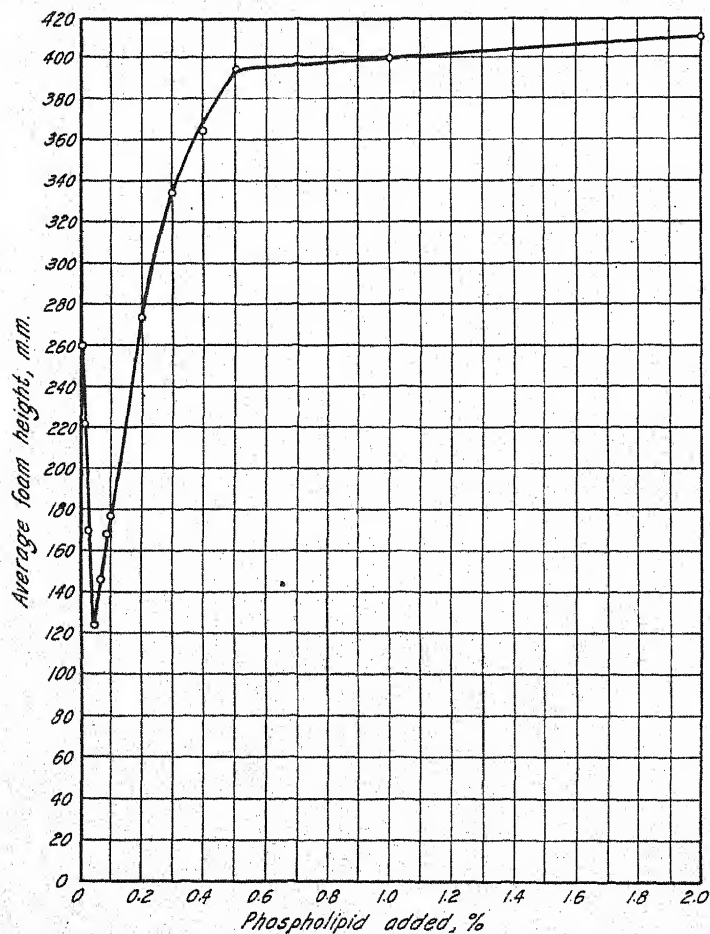


FIG. 4. The effect of added soybean phospholipids on the foaming of skim milk at 21.5° C.

of skim milk, gradually was replaced by a cream-type or lipoprotein-type foam.

No further increases in foaming capacity or foam stability occurred at temperatures above 50° C. In figure 6 it is seen that the emulsions made with fat containing about 0.8 per cent phospholipid yielded foams of minimum or low stabilities at all the temperatures below 50° C. The artificial

emulsions made with fat containing from 0.5 to 1.0 per cent phospholipid had foaming characteristics more closely resembling those of the natural emulsion made from cream and skim milk. At 23° C. the emulsions pre-

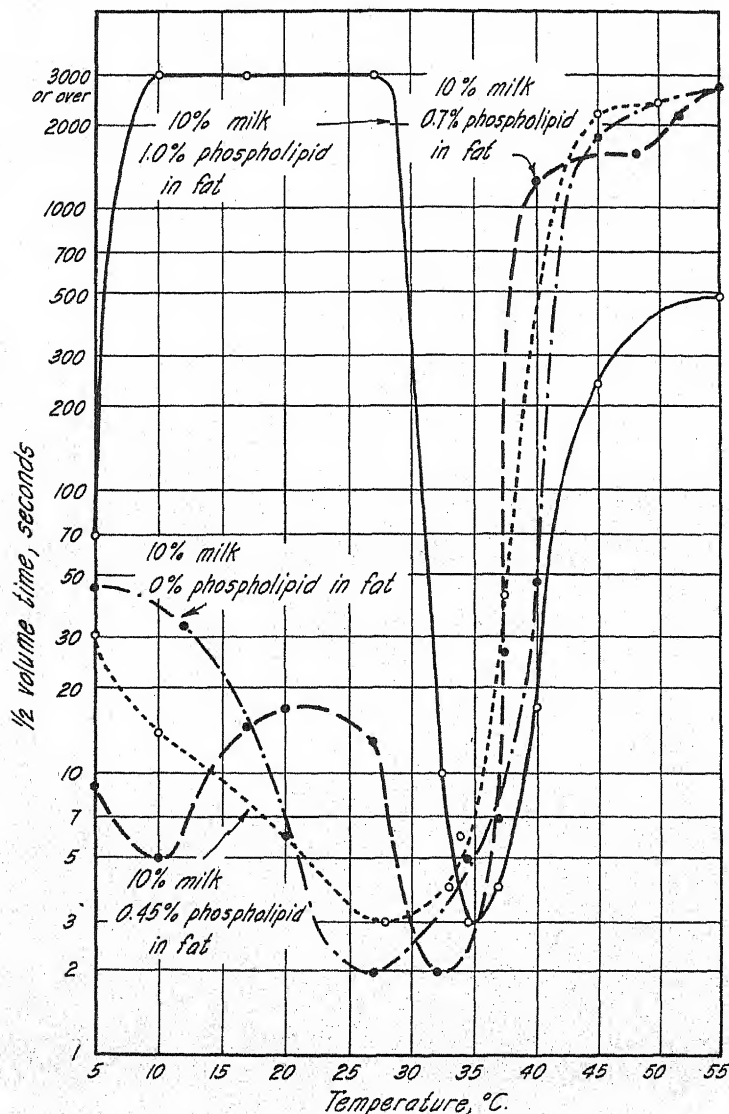


FIG. 5. The effect of temperature on the stability of foams of 10% emulsions of milk fat, containing varying concentrations of soybean phospholipids, dispersed in pasteurized skim milk.

pared with fat containing 0.8, 0.85, and 0.9 per cent phospholipid showed two types of foam, a protein foam that subsided quickly and the very stable cream-type of foam.



Effect of increasing the concentration of the fat of constant phospholipid content on the foaming of artificial creams. Artificial creams were prepared by emulsifying milk fat containing 0.5 per cent added soybean

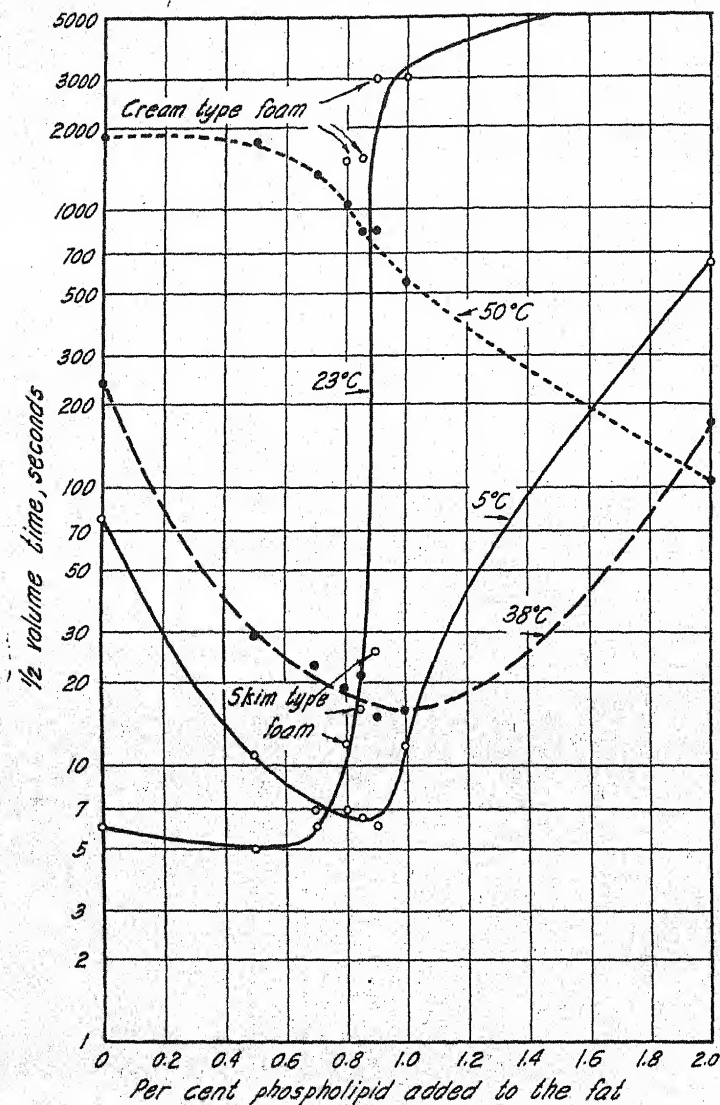


FIG. 6. The effect of increasing the phospholipid content of milk fat from 0.0 to 2.0% on the foam stabilities of a 10% emulsion in pasteurized skim milk at 5, 23, 38 and 50° C.

phospholipid into pasteurized skim milk. Natural creams were included. The results, shown in table 1, indicate that at 5 and 28° C. the foaming

tendency and the foam stability increase as the fat concentration increases from 10 to 40 per cent; the surface tension decreases with increasing fat concentration. At 50° C. the foaming capacity remains constant for increasing fat concentration, while the surface tensions and the half-volume times decrease progressively with increases in the fat. The surface tension values and the foaming properties of the 30 per cent artificial cream were very similar to those of the pasteurized natural cream of similar fat content.

*Effect on foaming properties of emulsifying milk fat, with and without added phospholipids, in buttermilk.* Wiese and Palmer (30) found that remade milk, made by dispersing butterfat in buttermilk, closely resembled whole milk in many respects, including the churnability of its cream. On

TABLE 1

*The effect of concentration of fat of constant phospholipid content on the foaming of artificial creams*

Sample	Results at 5° C.			Results at 28° C.		
	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
10% milk .....	.....	115	12	43.4	40	5
20% cream .....	.....	162	17	45.0	100	> 3000
30% cream .....	.....	200	1220	42.3	240	> 3000
40% cream .....	.....	205	> 3500	40.0	.....	.....
31% natural cream .....	51.0	200	> 3000	42.7	240	> 3000

the other hand, emulsions made by dispersing milk fat in calcium caseinate, lactalbumin, lactoglobulin, or phospholipid-aqueous media behaved quite abnormally. These investigators used milk fat in which the phospholipid presumably would be absent or nearly so. They did not add phospholipid to the fat.

Two creams were made by emulsifying milk fat or milk fat containing 0.5 per cent soybean phospholipid into buttermilk from sweet, pasteurized cream. Foaming was studied at three temperatures and the results compared with those obtained with a 31 per cent pasteurized natural cream. The results (table 2) show that the addition of phospholipid to the milk fat prior to emulsification improves the foaming properties of the artificial creams to the extent that they resemble those of natural cream. The foam stability of these creams at 5° C. and 23° C. is almost eight times that of the cream with no phospholipid added to the fat.

It appears to have been established that the natural fat globule "membrane" of milk and cream is some sort of phospholipid-protein com-

plex. Palmer (15) stated, "Indeed, not only was it found that the emulsion properties and churnability of artificial emulsions of milk fat in the various colloidal sols from milk plasma are strikingly different from those of washed natural cream but also that the 'membrane' materials isolated from the washed artificial creams are also chemically distinct from the natural 'membrane' substances". To the authors' knowledge no attention has been paid to the presence or absence of phospholipid dissolved in the fat being emulsified. It is known, however, that the amount of these compounds left associated with the fat after its isolation depends upon the method of

TABLE 2

*The foaming of 30% creams prepared by emulsifying butterfat, with and without 0.5% added soybean phospholipids, in buttermilk from sweet cream containing 31% fat<sup>a</sup>*

Cream	Results at 5° C.			Results at 23° C.		
	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
30% cream in buttermilk, no added phospholipid	54.4	200	396	48.2	220	435
30% cream in buttermilk, 0.5% phospholipid added to the fat .....	52.3	200	> 3000	43.5	240	> 3000
31% natural cream .....	51.6	200	3000	42.9	240	> 3000
Buttermilk (0.4% fat)	51.9	170	20	47.1	60	25

<sup>a</sup> At 50° C. all samples gave the protein-type foam.

isolation (6, 21). Jenness and Palmer (9) did have some indication that the protein as it is "eroded" during churning pulls away varying amounts of phospholipid with it. Observations in this laboratory (18) revealed striking visible differences at the interface between liquid milk fat superimposed upon a warm aqueous medium depending upon whether or not the fat contained phospholipid and whether or not the medium was pure water or contained whey proteins. The interface between the fat containing phospholipid and the pure water was cloudy, indicating solvation of the phospholipid; all the other interfaces remained clear.

*The effect of incorporation of phospholipids in the fat of artificial creams on some of their physical properties.* Soybean phospholipids were



dissolved in milk fat to a concentration of approximately 1.0 per cent by heating to 50° C. The solution was clear but showed a tendency to give an iridescent-type foam. A 25 per cent dispersion was prepared by emulsifying it in distilled water at 45° C. The emulsion was diluted to give creams containing 16.5 per cent butterfat, one-half being diluted with distilled water and the other half with a 0.5 per cent lactalbumin solution. The lactalbumin was isolated as described previously (4). Both creams were held overnight at 2° C.

TABLE 3

*Foaming properties of creams prepared with butterfat with 1.0% soybean phospholipid added emulsified in distilled water and in 0.5% lactalbumin solution*

Temp.	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	16.5% cream (1% phospholipid in fat) in distilled water			16.5% cream (1% phospholipid in fat) in 0.5% lactalbumin		
(°C.)	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
5	47	190	250	48.9	210	> 3000 <sup>a</sup>
16	40.9	40	10	46.6	185	1450 <sup>a</sup>
25	40.5	20	6	40.5	30	10
37	29.1	100	> 3000 <sup>b</sup>	29.6	15	5
45	29.1	105	> 3000	29.0	15	5
	Buttermilk from above cream 0.61% fat			Buttermilk from above cream 1.1% fat		
5	46.5	5	2	45.4	70	8
8	—	3	2	—	50	5
19	39.6	2	2	45.2	10	3
37	36.7	15	4	41.0	2	1
45	31.5	15	4	35.2	8	1.5

<sup>a</sup> Typical cream-type foam.

<sup>b</sup> At 37° C. the cream oiled off and an iridescent phospholipid foam originated from the oil layer on top.

Upon examination, the cream prepared in water contained large fat aggregates which stuck to the walls of the container and resembled those formed when normal cream is on the verge of "breaking" during churning. On heating to 37° C., the emulsion oiled off. Churning time at 10° C. was about 5 minutes. The butter formed a solid crumbly mass and the buttermilk contained 0.61 per cent fat (Babcock). The other cream prepared in the albumin solution had a smoother body, less tendency for fat aggregation, and a longer churning time (about 12 minutes). The butter was more

plastic, the butter granules maintained their individuality, and the buttermilk contained 1.1 per cent fat. The cream formed a stable cream-type foam between 5 and 15° C., and the foam decreased in volume and stability at increased temperatures.

Table 3 shows that the surface tensions of the creams were very similar except at the temperature of 16° C.; those of the buttermilk from the lactalbumin cream were higher than those of the other except at 5° C. The foaming characteristics of the creams and the buttermilks from them were diametrically different with respect to the effect of temperature. The lactalbumin-phospholipid stabilized cream gave the typical cream-type foam at low temperatures; the protein-free cream yielded the typical phospholipid foam at the higher temperatures.

TABLE 4  
*Phosphorus in the filtered butterfat from butter churned from artificial creams*

Sample no.	Description	Weight of fat ashed	Total phosphorus	Phosphorus
		(g.)	(g.)	(mg./g. fat)
1	Original fat	4.1527	0	0
2	Original fat plus approx. 1% phospholipids	4.0591	0.855	0.211
3	Fat from butter of cream from fat no. 2 in distilled water	4.0365	0	0
4	Fat from butter of cream from fat no. 2 in 0.5% lactalbumin solution	4.017	0	0

The analyses for phosphorus in the fat from the butter of each cream (table 4) showed that protein is not necessary to "pull" the phospholipid from the fat during churning. Apparently, the mere solvation of the polar phosphoric acid-choline group of the lecithin and of the phosphoric acid-ethanolamine group of the cephalin is sufficient.

#### DISCUSSION

The results of this study clarify, in some measure at least, the problem of making artificial emulsions of milk fat with properties similar to the natural product, using milk solids as stabilizers. Most workers in the past have overlooked the importance of incorporating phospholipids in the fat prior to emulsification. Wiese and Palmer (30) recognized that "The butterfat-in-buttermilk dispersion resembles whole milk in every respect, in general appearance, microscopic structure, cream separation and churnability of the cream." When they prepared stable emulsions with the

proteins of milk as emulsifiers, these emulsions were abnormal in one or more of their properties. The best churning was obtained with a phospholipid stabilizer. All stabilizers were incorporated in the aqueous phase prior to emulsification. Wiese *et al.* (29), in studies on the rebodding of cream, found that only those emulsions prepared with the normal fat globule membrane present during the emulsification responded to the rebodding process. They also noted that the composition of the butterfat appears to be a factor, presumably referring to the chemical constants of the fat, rather than its purity with respect to phospholipids. As stated earlier, the phospholipid content of filtered milk fat depends upon the purification procedure (6, 21). It is known also that the phospholipids of milk, cream, and butter exist in at least three states: free, loosely-bound to protein (probably by secondary valences), and chemically-bound to protein (primary valences) (2, 17, 26). Jenness and Palmer (9) emphasized that the ratio of phospholipid to protein was greater in the serum of washed-cream butter than in its buttermilk. It would be interesting to know the ratio of cephalin to lecithin in this respect, especially in view of the work of Spiegel-Adolph (25), Chargaff (2) and Rewald (17), who showed that the phosphatides of butter consisted of 50 per cent lecithin, 36 per cent cephalin, and 14 per cent other phosphatides.

Exact duplication of the foaming or other physical properties of a milk or cream by using artificial emulsions compounded from purified milk fat and other milk solids isolated by chemical means obviously is extremely difficult. The natural membrane material from cream which has not been washed too well would be expected to be the best emulsifier.

The authors postulate the following explanation, based on thermodynamical considerations. Assuming that milk fat is elaborated separately from the plasma solids (16), that the neutral blood fat is the main precursor of milk fat, and that blood phospholipids or phospholipid-protein complexes take part in its transfer, it seems logical to assume that, initially, milk fat contains, or is associated with, phospholipids, free and/or linked to protein. These, being surface-active, would tend to migrate to the interfaces between the fat and the aqueous medium during globule formation, their relative concentration being proportional to their surface activity. The phosphoric acid-choline polar group of lecithin and the phosphoric acid-ethanolamine polar group of cephalin would orient themselves toward the aqueous phase. They would become solvated in pure water, but, in the presence of plasma proteins, functional groups of both phospholipids and proteins likely would react through primary valences to form salt-like compounds. It is known that lecithin and cephalin form complexes with bacterial cells which inhibit the action of synthetic detergents (1). Cephalin has been shown to react with serum albumin and salmine, the rate of reaction being high; lecithin appears to react more slowly (2). Other



less stable complexes are possible through secondary valences or adsorption. Macheboeuf and Sandor (13) voiced similar ideas in connection with blood.

This speculation supports the theory advanced by Rimpila and Palmer (20), *viz.*, "It appears possible that the 'membrane' may be formed before the fat globules become a part of the milk or that the fat globules may be secreted before the milk plasma is completely formed, in which case the 'membrane' materials could be considered, in part at least, as precursors of plasma materials." The speculation is not counter to the idea of a special 'membrane' protein, or to the finding that the membrane protein of cream washed four times with water, four times with rennet whey, and then four times with water, and that of an artificial whey cream washed three times with water, have a sulfur content practically identical with the natural membrane. The membrane itself was lower in lipid phosphorus when whey was not used (20). The speculation is in keeping with the lipid extraction data of Tayeau (26), who found 20 per cent of the phospholipids in milk extractable with ether, 30 per cent with ether and soap, and the remainder extractable by ether after treatment with boiling alcohol to denature the protein in combination with the phospholipids.

As an outgrowth of these studies the authors have adopted the practice of incorporating phospholipids in the fat as well as in the whey, skim milk, or other media in making remade milks or creams, providing the media do not already contain appreciable phospholipid or the lipoprotein complex; buttermilk requires none. The principle has found industrial application in making wartime substitutes for ice cream and also genuine ice cream (28). Lecithin, for example, is separately incorporated into both the aqueous and fatty phases. Josephson and Dahle (10) succeeded in imparting normal whipping properties to ice cream mixes made with butter or butter oil as the source of fat by emulsifying the fat with either dried egg yolk or the natural "membrane suspension" before incorporating the fat into the mixes. These authors considered that a protein-phospholipid complex, already formed, was essential for proper emulsification; specificity was allotted to the true "membrane" protein moiety of the complex. Sell *et al.* (23), in studies with mayonnaise, showed that the lecitho-protein complex of egg yolk was the emulsifying agent in egg yolk, whereas free lecithin and free cephalin were detrimental. The results of the present study would seem to indicate that unaltered serum proteins with functional groups available for combining with functional groups of the phospholipids are adequate for the formation of such complexes.

#### SUMMARY

The role of milk fat in the foaming of milk, cream, buttermilk, and their artificial counterparts has been studied at temperatures between 5 and 50° C. The results suggest the following conclusions.

1. In such emulsions two types of foam may appear separately or simultaneously, a protein type and a phospholipid-protein type. At the higher temperatures the protein type predominates.

2. Whole milk, cream, and buttermilk exhibit minimum foaming at 30-35° C.

3. At 35° C. the foam volume and the foam stability of skim milk are decreased as the fat content is increased up to about 5.0 per cent. With further increases in the fat content both the volume and stability of the foam increase up to a fat content of 20 per cent, after which no further increases occur. At 6° C. the foam volume remains constant regardless of the fat content. The stability of the foam reaches a minimum at about 5 per cent fat concentration, after which it increases rapidly until a fat content of 10 per cent is reached, above which cream-type foams of high stability are formed.

4. Artificial milks and creams were made to resemble the natural product only when phospholipids (soybean) were incorporated into the fat prior to emulsification. The medium should be a protein sol; a lactalbumin sol or a milk serum protein sol such as rennet whey was satisfactory. The most normal cream was made when the medium contained natural fat globule "membrane" material; buttermilk met this condition.

5. Emulsions of pure milk fat in skim milk, gelatin, or gum arabic sols have abnormal foaming properties.

6. For emulsions with fat dispersed to a degree comparable to that of natural milk or cream, the optimum concentration of mixed phospholipids in the fat appears to be from 0.8 to 1.0 per cent. Unbound phospholipid appears undesirable.

7. These results have been discussed as they apply to churning, to cream whipping, to cream rebodilyng, and, by inference, to ice cream whipping.

#### REFERENCES

- (1) BAKER, Z., HARRISON, R. W., AND MILLER, B. F. Inhibition by Phospholipids of the Action of Synthetic Detergents on Bacteria. *J. Exptl. Med.*, 74: 621-637. 1941.
- (2) CHARGAFF, E. The Protamine Salts of Phosphatides, with Remarks on the Problem of Lipoproteins. *J. Biol. Chem.*, 125: 661-670. 1938.
- (3) COLE, W. C., AND SMITH, F. R. A Microscopic Technique for Studying Fat Globules in Dairy Products and Other Oil in Water Emulsions. *J. Dairy Sci.*, 22: 420-421. 1939.
- (4) EL-RAFEY, M. S., AND RICHARDSON, G. A. The Role of Surface-active Constituents Involved in the Foaming of Milk and Certain Milk Products. I. Milk Proteins. *J. Dairy Sci.*, 27: 1-18. 1944.
- (5) EL-RAFEY, M. S., AND RICHARDSON, G. A. The Role of Surface-active Constituents Involved in the Foaming of Milk and Certain Milk Products. II. Whey, Skimmed Milk and Their Counterparts. *J. Dairy Sci.*, 27: 19-31. 1944.

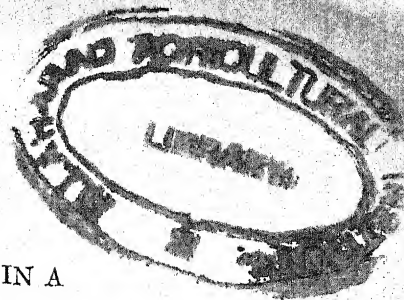


- (6) EL-RAFEY, M. S., RICHARDSON, G. A., AND HENDERSON, J. L. The Effect of the Method of Manufacture of Butteroil on Its Keeping Quality. *J. Dairy Sci.*, 27: 807-820. 1944.
- (7) HOLM, G. E. Chapt. VII in *Fundamentals of Dairy Science*. Associates of Rogers. 2nd Ed. Reinhold Publ. Corp., New York, N. Y. P. 191. 1935.
- (8) HORRALL, B. E. A Study of the Lecithin Content of Milk and Its Products. *Ind. Agr. Expt. Sta. Bull.* 401. 1935.
- (9) JENNESS, R., AND PALMER, L. S. Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. V. Composition of the "Membrane" and Distribution of the Adsorbed Substances in Churning. *J. Dairy Sci.*, 28: 611-623. 1945.
- (10) JOSEPHSON, D. V., AND DAHLE, C. D. The Importance of the Fat Globule Membrane in the Freezing of Ice Cream. *Rept. Proc. Intern. Assoc. Ice Cream Mfrs.*, 36th Ann. Conv., 2: 100-115. 1936.
- (11) LEETE, C. S. Foaming of Milk and Cream. *U. S. Dept. Agr. Circ.* 108. 1930.
- (12) LEVITON, A., AND LEIGHTON, A. The Action of Milk Fat as a Foam Depressant. *J. Dairy Sci.*, 18: 105-112. 1935.
- (13) MACHEBOEUF, M. A., AND SANDOR, G. Recherches sur la Nature et la Stabilité des Hasons Protéides—lipides du Serum Sanguin. Étude de l'extraction des lipides par l'éther en présence d'alcool. *Bull. soc. chim. biol.*, 14: 1168-1190. 1932.
- (14) MOHR, W., AND BROCKMANN, C. Beitrage zum Schaumproblem. *Milchw. Forsch.*, 11: 48. 1930.
- (15) PALMER, L. S. The Structure and Properties of the Natural Fat Globule "Membrane". *J. Dairy Sci.*, 27: 471-481. 1944.
- (16) PETERSEN, W. E., PALMER, L. S., AND ECKLES, C. H. Synthesis and Secretion of Milk Fat. *J. Dairy Sci.*, 90: 573-581. 1929.
- (17) REWALD, B. Les Phosphatides dans le Résidu du Beurre. *Lait* 19: 22-25. 1939.
- (18) RICHARDSON, G. A. Unpublished data. University of California, Davis. 1942.
- (19) RICHARDSON, G. A., TARASSUK, N. P., AND FRY, L. B. The Manufacture of Non-foaming Casein (Abs.). *J. Dairy Sci.*, 20: 449. 1937.
- (20) RIMPILA, C. E., AND PALMER, L. S. Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. IV. Factors Influencing the Composition of the Adsorption "Membrane". *J. Dairy Sci.*, 18: 827-839. 1935.
- (21) RITTER, W. The Foaming of Fats, Especially Butter, When Used for Baking. *Mitt. Lebensm. Hyg.*, 29: 253-261. 1938. (Abs. *J. Dairy Sci.*, 22: A96. 1939.)
- (22) SANMANN, F. P., AND RUEHE, H. A. Some Factors Influencing the Volume of Foam on Milk. *J. Dairy Sci.*, 13: 48-63. 1930.
- (23) SELL, H. M., OLSEN, A. G., AND KREMERS, R. E. Lecitho-Protein. The Emulsifying Ingredient in Egg Yolk. *Ind. Eng. Chem.*, 27: 1222-1223. 1935.
- (24) SOMMER, H. H., AND HORRALL, B. E. Ann. Rept. of the Director. *Wis. Agr. Expt. Sta. Bull.* 410. Pp. 25-27. 1930.
- (25) SPIEGEL-ADOLPH, MONA. Physical Chemistry of Lipoids. II. The Protective Power of Cephalin. *J. Am. Chem. Soc.*, 57: 1431-1433. 1935.
- (26) TAYEAU, F. Recherches sur l'état physiochimique des diverses Substances lipoidiques du lait et particulièrement des Phosphatides et du Cholestérol. *Lait*, 20: 129-134. 1940.
- (27) VAN DAM, W. Opstellen over moderne Zuivelchemie. 2nd Ed. Gravenhagen, Netherlands. Pp. 199-218. 1922.



- (28) WALKER'S DAIRIES LTD., AND HUMPHRISS, E. L. E. Improvement in Emulsions Suitable for Ice Cream or the Like. Brit. Pat. 552,561. (Cited from Dairy Sci. Abs., 5(3): 120. 1943.)
- (29) WIESE, H. F., NAIR, J. H., AND FLEMING, R. S. Improvement in the Viscosity of Pasteurized Cream through Subsequent Heat Treatment. J. Dairy Sci., 22: 875-881. 1939.
- (30) WIESE, H. F., AND PALMER, L. S. Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. I. The Churnability of Artificial Emulsions Prepared with the Known Emulsifying Agents of Cream. J. Dairy Sci., 15: 371-381. 1932.
- (31) YOE, J. Photometric Chemical Analysis. Vol. I. John Wiley and Sons, New York, N. Y. Pp. 348-351. 1928.

1000



## A STUDY OF MULTIPLE BIRTHS IN A HOLSTEIN-FRIESIAN HERD<sup>1</sup>

K. O. PFAU, J. W. BARTLETT, AND C. E. SHUART  
*Department of Dairy Industry, College of Agriculture,  
Rutgers University, New Brunswick, New Jersey*

Dairy cows are uniparous animals. Multiple births are relatively rare and occur with variable frequency. Twin births are most frequent, and triplets, quadruplets, and quintuplets are progressively rarer. Numbers of twin births reported for individual dairy breeds range from less than 0.5 per cent to 4.5 per cent (11) and for individual herds up to 8.8 per cent (9).

There still are differences of opinion as to whether twin births are desirable or undesirable in dairy cattle breeding (4, 5). Hewitt (7) considers multiple births a sign of increased fecundity and fertility, whereas Williams (12) relates such births to unsound or even diseased conditions of the genital tract, in particular of the ovary.

A general study of fertility in dairy cows, in which twin births were recognized as one of the factors influencing reproductive performance, led to a more detailed investigation of multiple births. The results are presented herewith.

### SOURCE OF DATA

The data for this study were taken from the records of the Holstein-Friesian experimental herd at the New Jersey Agricultural Experiment Station and cover a period of about 15 years. The breeding program and operations pursued in this herd are rather unique insofar as the herd is self-containing and inbreeding is practiced to a high degree. The inbreeding with rigid selection is manifested in the preservation and concentration of the young animals' relationship to the noted sire, Ormsby Sensation 45th. In publications of Bartlett and Margolin (1) and Bartlett *et al.* (2, 3) detailed accounts are given of the conduct and progress of this experimental breeding project. From these reports it is evident that the artificial selection was not specifically directed toward reproductive efficiency and twinning but toward such qualities as milk and butterfat production, butterfat percentage, and body conformation.

### RESULTS

By nature of the character investigated, the results must be so evaluated

Received for publication November 6, 1947.

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Industry, New Brunswick.



that small changes in actual numbers could have notable effect upon some of the summaries and averages.

*General occurrence of multiple births.* From 1931 to 1946 there were 937 parturitions and abortions available for observation. Of these, 37 or 3.95 per cent were twin births and 2 or 0.21 per cent triplets, making a total of 39 or 4.16 per cent multiple births. In other words, the average incidence of twin births was one in every 25.3 and triplets one in every 468.5 births, or one multiple birth in every 24.0 births. The 39 multiple births were observed in 36 cows; three cows gave birth to twins twice. Since only two sets of triplets occurred and the gestation of one set ter-

TABLE 1  
*Multiple births in relation to age of dam*

Age by parturition no.	Observed parturitions	Observed multiple births	
	(no.)	(no.)	(%)
1	269	2	0.74
2	199	10	5.03
3	142	6	4.23
4	100	5	5.00
5	77	6	7.79
6	51	5	9.80
7	41	3	7.32
8	30	0	0
9	23	1	4.35
10	4	1	25.00
11	1	0	0
Summary	937	39	4.16

minated in an abortion without sex determination of the fetuses, it was considered advisable in these investigations to include the triplets along with the twin births.

*Twin births in relation to age of dam.* Frequency of twinning in relation to age of dam is summarized in table 1, where age is expressed in parturition numbers. The first parturition occurred at an average age of about 29.5 months, and the average interval between parturitions was about 14.5 months. This tabulation shows that the number of twin births has been extremely low at the first parturition. From then on it increased with age at calving, first increasing abruptly at the second parturition and then gradually reaching a peak at the fifth, sixth, and seventh par-

turations. Above these ages a decrease seemed to take place, but in view of the small number of cases not too much weight should be given to this observation.

*Length of twin gestations.* Confirming Hewitt's findings in British Friesian cows (7), twin gestations in this herd were of shorter duration than gestations of single calves. The highly significant difference amounted to about 7 days. Between the sexually unlike twin pairs, differences also existed, as shown in table 2. The mean length of gestation of the male pairs was greater than that of the sexually-mixed pairs and considerably greater than that of the female pairs. It appears, as in the case of single calves (10), that sex of the fetuses has an influence upon length of gestation.

*Sex ratio.* The sex ratio of 36 twin and one triplet births was found to be 33 males to 42 females, or 44 per cent males. The theoretical ratio

TABLE 2  
*Length of gestation of twins*

	No. of gestations observed	Length of gestation	
		Mean days	Standard deviation days
Male pairs .....	6	275.33 $\pm$ 3.97	6.87 $\pm$ 1.98
Sexually-mixed pairs .....	11	273.64 $\pm$ 2.74	8.23 $\pm$ 1.75
Female pairs .....	14	270.00 $\pm$ 2.56	8.86 $\pm$ 1.68
Summary .....	31	272.99 $\pm$ 1.68	9.37 $\pm$ 1.19

of twin pairs, 1 ♂♂ : 2 ♂♀ : 1 ♀♀ was met by an actual one of 9 ♂♂ : 13 ♂♀ : 14 ♀♀, or 1.00 : 1.44 : 1.56. Although these ratios are rather unusual, their differences from the theoretical are not statistically significant.

*Vitality of multiple calves.* The vitality of multiple calves was evaluated according to the number born dead or that died within 2 days after birth and by following up the individual life histories of female twin pairs.

The mortality rate at birth for each sex is presented in table 3. It was slightly higher for male calves than for females. The average for both was 22.67 per cent, in contrast with only 9.65 per cent for the whole herd.

The history of the 14 female twin pairs revealed that 6 individuals, or 21.43 per cent, were born dead or died soon after birth; 5, or 17.86 per cent, were sold when immature, mostly for reasons of selection; 6, or 21.43 per cent, freshened in the herd but showed relatively poor production and breeding records; only 3, or 10.72 per cent, remained in the herd.

for the length of their natural life, averaging 8.5 years, and exhibited relatively good production and breeding records; and 8, or 28.57 per cent, still in the herd at the time of this writing were immature, ranging in age from 4 to 18 months.

The mortality rate and life history of these heifer calves strongly indicate that twins have a lower vitality and poorer prospects of productive life than single calves.

*Retained placentae and twin births.* The condition of retained placentae after twin births was very much aggravated when compared with births of single calves. Of 31 apparently normal multiple parturitions, 23, or 74.19 per cent, were accompanied by this condition. The percentage for the whole herd was 23.10.

*Conception rate of dams after twin births.* After giving birth to twins, the dam's conception rate for a succeeding pregnancy should be another

TABLE 3  
*Mortality of multiple calves*

	Males	Females	Total
No. of calves .....	33	42	75
Dead and aborted calves .....	8	9	17
Per cent mortality .....	24.24	21.43	22.67

indication of the possible effect of twinning on future breeding efficiency. An analysis of the records revealed that 71 services were required for 22 safe pregnancies in as many cows after twin births, corresponding with a conception rate of 3.23. This conception efficiency was almost 50 per cent lower than that of the herd average, which amounted to 2.21.

*Calving interval after twin births.* After the 37 twin births, apparently normal pregnancies and parturitions were noted in 13 instances. The mean calving interval was  $483.5 \pm 22.3$  days. This interval corresponded with a breeding efficiency of 75.41 per cent, which was considerably below the herd average of 82.59 per cent. This difference alone suggests that twin births cause a reduction in reproductive efficiency.

*Influence of twin birth upon future reproductive performance.* In the 39 cases of multiple parturitions, 15 cows, or 38.46 per cent, continued to produce in apparently normal fashion, except for the higher conception rate and longer calving intervals. On the average, these cows survived their twin calving age by 2.33 parturitions. Five cows, or 12.85 per cent, became sterile; 12 cows, or 30.77 per cent, were sold for various reasons shortly after giving birth to twins; and three cows, or 7.69 per cent, died after the twin parturitions, one because of hardware, another,



from a ruptured uterus, and the third from an unknown cause. Of the four remaining cows which have given birth to twins within the last 9 months and which are still in the herd, two already have exhibited breeding troubles.

This recorded information does not warrant definite conclusions in regard to the influence of twin births on the future reproductive performance of the cows. The five proved cases of sterility are not in excess of the expectation for the whole population. It must be remembered that the disposals include a number of cases which must be regarded as doubtful in this respect. On the other hand, the first group, which consists of the 15 cows with an apparently normal reproductive performance after twin births, comprises the largest proportion of the grouped twin dams. Their performance at least implies that twin births do not necessarily cause breeding troubles with sterility implications or shorten the reproductive life of the cows.

*Breeding efficiency of twin dams.* Previously it was shown clearly that twin births exert a depressive effect upon the dam's subsequent

TABLE 4

*Breeding efficiency of twin dams compared with the herd as a whole*

Group	No. of cases	Percentage of breeding efficiency			
		Mean	Standard deviation	Coefficient of variation	Skewness of distribution
Herd as a whole	144	83.99±0.94	11.32±0.66	13.48	+0.0483
Twin dams .....	26	83.46±1.59	7.96±1.10	9.54	-0.0302

breeding efficiency. That this will affect a cow's lifetime breeding efficiency in proportion to her life span is acknowledged for the following analysis.

The lifetime breeding efficiency was determined for 26 twin dams, the only ones with complete records available. Their mean breeding efficiency and the standard deviation and coefficient of variation, as well as the approximate measure of skewness, were compared with the respective values of the herd as a whole.

Table 4 shows that almost no difference existed in the mean breeding efficiency between these two groups. The values for the standard deviation and coefficient of variation were considerably smaller for the twin dams than for the whole herd, denoting a greater uniformity of the twin group. Moreover, the distribution of the twin group was skewed positively, that is, toward the higher values, while the distribution of the whole herd was skewed negatively. Since twinning in itself has a depressing effect

upon the breeding efficiency, as already established, and since the frequency distribution of the herd as a whole markedly is skewed toward the lower values, a strong argument is offered for a fundamentally higher breeding efficiency in favor of the twinning group.

*Milk production of twin dams.* A study of the relationship between twinning and milk production was another object of this investigation. For this purpose the records of the 26 twin dams already employed in the analysis for breeding efficiency were considered suitable for a comparison of the milk production between twin dams and the herd as a whole.

The milk yield in both groups was expressed in pounds of 4 per cent fat corrected milk (5) per day on a mature equivalent twice-a-day milking basis. The actual milk yield was converted to the mature equivalent by the use of conversion factors based on the production records of the herd itself. The daily average was calculated on the total adult days the individual cow stayed in the herd, starting at the age of 27 months and continuing until her last calving.

TABLE 5  
*Milk production of twin dams compared with the herd as a whole*

Group	No. of cases	Milk yield in lb.			
		Mean	Standard deviation	Coefficient of variation	Skewness of distribution
Twin dams .....	26	30.08 $\pm$ 1.06	5.32 $\pm$ 0.74	17.70	— 0.7080
Herd as a whole	144	29.10 $\pm$ 0.59	7.11 $\pm$ 0.42	24.42	— 0.3047

The results are presented in table 5. The constants chosen for this comparison were the same as those used for the analysis of breeding efficiency. The mean milk yield was insignificantly in favor of the twin dams ( $P = 0.70$ ). The standard deviation and the coefficient of variation of the twin dams were considerably smaller than those of the herd, indicating greater uniformity for the first group. The frequency distributions of both groups were skewed negatively. This analysis indicated that the twin dams were at least equal in milk production to the herd as a whole. Superiority on this basis alone could not be demonstrated.

*Comparison of various characteristics of fertility in twin dams and in the whole herd.* Although the results of some investigations (7) indicate that twinning in dairy cows is associated with high reproductive qualities, this question is far from settled. Few data are available for correct evaluation of fertility itself, much less in relation to twinning. Thus, although twinning may be an expression of female fecundity in itself, the structural form of the female reproductive tract seems to obstruct the expression.

This conflict could be noted in the records and investigational results already cited and might interfere with most comparisons.

Besides breeding efficiency, the following criteria were used in the evaluation of reproductive performance: Live and dead calves born, twin births, abortions, and retained placentae. The comparison between twin dams and a representative group of cows producing only single calves is shown in table 6.

From this tabulation it will be seen that with the exception of the per cent of live calves, these measures of fertility all were in favor of the cows giving birth to single calves. That the greater percentage of living calves

TABLE 6  
*Comparison of various measures of fertility  
between twin dams and other herd representatives*

	Twin dams	Herd representatives
Total no. of cows observed .....	28	142
Av. coefficient of inbreeding per cow .....	0.09	0.09
Av. gestation number observed .....	3.73	3.12
Av. conception rate .....	2.53	2.10
Based on total no. of parturitions:		
% of live calves .....	100.00	83.35
% of dead calves .....	17.50	7.83
% of multiple births .....	23.23	0.0
% of abortions .....	9.17	6.57
% of retained placentae .....	30.83	18.69

for the twin dams' group was due almost exclusively to the twin births was revealed simply by adding the percentages of live and dead calves in both groups and subtracting the percentage of multiple births from the twin dams' group. Should allowance be made for the lack of vitality of twin calves, this slight superiority would vanish.

Another important point in this tabulation is the average observed gestation number, which is considerably higher for the twin dams. Since the gestation numbers stand in relation to the average age at calving of the cows, their averages indicate strongly that the twin dams were older than the cows with only single calves. Two factors might have contributed to this effect. Probably the main factor was the expression of twinning relatively late in life, making the twin dams a selected group in this respect. Many of the younger cows in the second group were potential twin dams. The other factor would be that twin dams actually were longer lived.



*Twinning and inbreeding.* When twinning was set in contrast with the degree of inbreeding of the cows in the herd, as illustrated in table 7, a non-uniform positive trend between these two characteristics was observed.

This feature does not mean that twinning was dependently related to the degree of inbreeding as such. It indicated rather well, however, that the factors for the twinning disposition were present in some of the foundation animals. By directing the breeding operations to the inbreeding of such animals, these factors became more concentrated in some cows and expressed themselves more often than in the foundation cows.

*The hereditary aspect of twinning.* Heredity control of multiple births in mammals has been proved amply in sheep, goats and other animals. In

TABLE 7  
*Comparison of inbreeding with twinning*

Group	Coefficient of inbreeding	No. of cows	No. of parturitions	No. of twin births	% of twin births
1	0.00	55	116	2	1.72
2	0.01-0.04	92	291	10	3.44
3	0.05-0.09	21	67	0	0.0
4	0.10-0.14	36	87	4	4.60
5	0.15-0.19	29	73	7	9.59
6	0.20-0.24	9	16	1	6.25
7	0.25-0.29	10	22	1	4.55
8	0.30-0.39	3	8	1	12.50
Total		255	680	26	3.82

dairy cows this proof is attained only with difficulty, because its relatively rare appearance and its dependence upon the dam's age frequently hide the presence of this character. The small number of offspring in dairy cows and, possibly, environmental influences upon twinning contribute to the difficulties. Statistical investigation of the problem would necessitate large numbers of reliable and complete records such as those accumulated in herd book organizations. Unfortunately, these generally lack completeness, because only promising offspring are reported. An alternative, used in the present study, is the investigation of individuals and family groups in large herds where complete records are kept over long periods.

In table 8 the occurrence of twin births by parturition numbers is summarized for the members of 21 cow families which make up over 90 per cent of the present herd. Cows of ten of these families never had any

twins recorded. In the remaining 11 families the rate of twin parturitions ranged from 2.63 to 18.18 per cent.

In table 9 the twin parturitions of the daughters of 19 sires are presented. These daughters are, for the most part, the same cows represented in the cow families listed in table 8. In grouping these cows according to their sires, it was found that nine sires did not have any daughters with twin births. The remaining ten sires had one or more daughters which gave birth to twins. On the basis of all parturitions of the daughters of

TABLE 8  
*Occurrence of twins by cow families*

Cow family no.	No. of parturitions	No. of twin pairs	% of twin pairs
3	20	0	0.0
15	17	1	5.88
20	18	0	0.0
37	23	1	4.35
61	66	6	9.09
64	22	4	18.18
66	48	2	4.17
68	20	0	0.0
69	21	1	4.76
75	14	0	0.0
78	20	1	5.00
80	26	0	0.0
82	34	2	5.88
91	7	0	0.0
92	38	1	2.63
93	24	1	4.17
95	17	0	0.0
96	14	2	14.29
97	15	0	0.0
100	13	0	0.0
103	25	0	0.0
Total	502	22	4.38

these individual sires, the ten daughter groups varied from 2.27 to 14.29 per cent in twin births.

This variation in both groups might be attributed to three sources, namely, pure chance, environment, and the genetic twinning disposition of sires and dams. The emerging combination of the daughters' germ plasm founded upon the physical basis of heredity was the determining principle of this disposition. Environmental factors such as feeding and management probably were of very minor influence. Hormonal therapy, though of considerable importance in twinning, as shown by Hammond and Bhattach-

arya (6), was of no consequence in this herd. With the generally low percentage of twin births, the expression of the disposition was very uncertain and the chance factor could not be discounted.

By means of genealogical diagrams of cow families that have had a relatively high number of twin births it can be demonstrated that the disposition for twinning is inherited.

Figure 1 is a genealogical diagram of cow family 61. It is arranged with the foundation cow on top, her female progeny following down the

TABLE 9  
*Occurrence of twins by sires' daughters*

Sire no.	No. of daughters included	No. of parturitions	No. of twin pairs	% of twin pairs
A	6	20	0	0.0
B	4	10	0	0.0
C	13	44	1	2.27
D	4	10	0	0.0
E	19	62	3	4.84
F	8	41	1	2.44
H	3	8	1	12.50
I	18	68	3	4.41
L	6	21	0	0.0
N	15	41	2	4.88
O	27	77	7	9.09
R	4	5	0	0.0
T	32	80	2	2.50
U	18	35	2	5.71
V	7	10	0	0.0
W	5	11	0	0.0
X	6	14	2	14.29
D-1	3	8	0	0.0
F-1	3	4	0	0.0
Total	201	569	24	4.22

line, generation by generation. The individual offspring is designated by herd number. After the cow's number is given in parentheses the number of her twin parturitions, if any. For the younger offspring alive in the herd at this writing, the letter *P* for prospect was added. Animals that are twins themselves are so designated by numbers in bold-face type. Below the female's identification number is ascribed the sire's identification, generally by a letter or letter with number. Beyond that, separated by a dash, the percentage of twin births of the sire's daughters is given as far as it is known.

The figure illustrates how the transmission of the disposition for twin-



ning may work out in a relatively large cow family. Foundation cow 61, herself a twin, was sired by bull *C*, whose daughters born in the herd showed only 2.3 per cent twinning. Cow 61 had five daughters with reproduction records. They were sired by three different bulls, making three of them full sisters by sire *E*, whose daughters averaged 4.8 per cent twin births. Their records and especially those of the three full sisters suggest strongly a segregation in the Mendelian ratio. Two of the three full sisters, namely nos. 67 and 294, started progeny lines of their own. Neither of these lines exhibited any twinning. The third full sister, no. 256, produced one pair of twins in her second parturition. Unfortunately, she did not leave any progeny in the herd. Her early expression of the twinning character suggests, however, that she was highly predisposed to it.

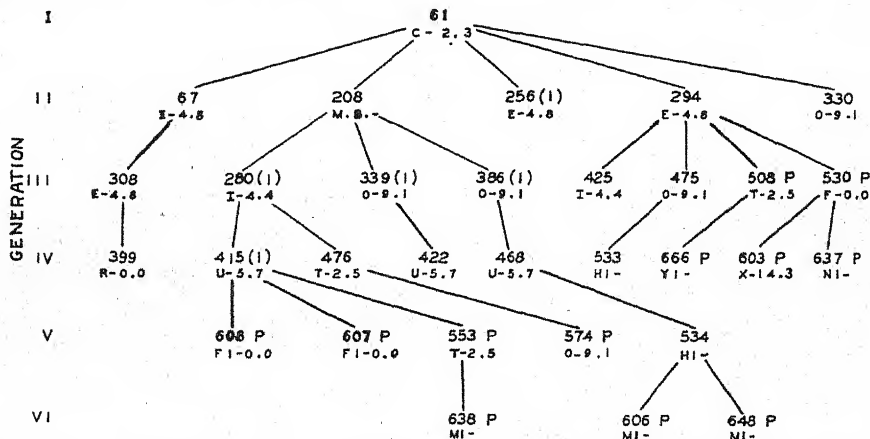


FIG. 1. Diagram of cow family 61 showing the extent and distribution of twin births, which amount to 9.09 per cent of all observed parturitions. For explanations of the diagram see text.

Daughter no. 208, which was sired by a bull designated *M. B.* and for which no twinning record could be established because of lack of daughters in the herd, started the longest line of progeny. Though no. 208 herself revealed no twinning disposition, her progeny visibly exhibited it to an extreme degree. Every one of her three daughters gave birth to one pair of twins. In turn, their progeny (generation IV) again suggest that Mendelian segregation might have been at work. The animals listed under generations V and VI are too young at present to allow any conclusion.

The fifth daughter, no. 330, of foundation cow 61 was sired by bull *O*, which was by progeny test a highly predisposed animal. Cow 330 calved only twice in the herd and produced single bull calves. Her disposition for twinning, therefore, never will be ascertained.

A similar investigation was made on cow family 64; her genealogy is diagrammed in figure 2. Family 64 was relatively small. Over a period covering five generations, this family was just about holding its own. In respect to twinning, this family's record, amounting to 18.18 per cent, was higher than that for any other cow family observed. The distribution of twin births extended over only three generations. The foundation cow did not visibly express any twinning disposition and the descendants in the fifth generation were too young to show any. A segregation into unusually predisposed and undisposed lines was hinted in this family, a feature closely resembling that of family 61.

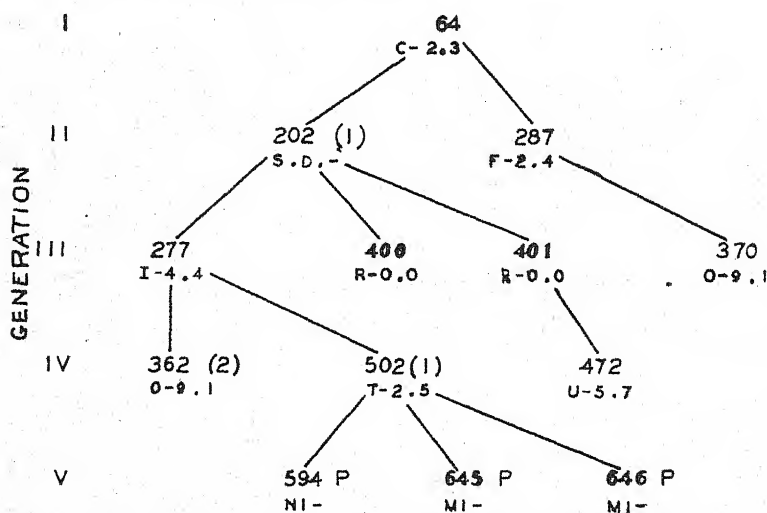


FIG. 2. Diagram of cow family 64 showing the extent and distribution of twin births, which amount to 18.18 per cent of all observed parturitions. For explanations of the diagram see text.

In both families virtually the same bulls appear as sires to the progeny. Sire *O*, with a high average twinning rate, is especially conspicuous as a sire of twin dams; so also is sire *I*, with a relatively low average rate. Both these sires are inbred to bull *C*, which is also the sire of both foundation cows. Sires *E* and *F*, both outbreds, are conspicuously present in the non-predisposed lines.

Obviously, the low frequency of twin births does not warrant definite conclusions with respect to the mode of transmission of twinning. The continued use of inbred sons and grandsons of bull *C* for several generations in succession and with it a concentration of certain genes of this bull suggest the possibility that he carried genes which foster twinning. In addition, it is observed that other inbred sons and grandsons of the same bull, as sires *T* and *R*, have very low rates for twinning. It should be

recalled that almost half of the cow families and half of the sires never showed any tendency for twinning. All of these observations point strongly to the conclusion that twinning in dairy cows is influenced chiefly by heredity yet expresses itself differently with age. The mode of transmission could well be understood by the assumption that twinning is under the control of a small number of autosomal genes which express themselves incompletely. The transmission seems to be recessive in character, with gene interactions or modifications.

#### DISCUSSION AND CONCLUSIONS

The observation made in this investigation that twinning is rare in the first parturition, rises to a peak in the fifth, sixth, and seventh parturitions, and then decreases with advancing age harmonizes closely with the general cycle of fecundity in most multiparous mammals. From this observation alone it is most probable that twinning is an expression of fecundity or the potential reproductive capacity of a dairy cow. However, if dairy cows are considered as strictly uniparous animals, and they should be so considered according to the structural development of their reproductive tract, twinning could be regarded as cases of reversion or atavism.

Evidence was presented that twinning is chiefly controlled by heredity. The effect of environment seems to be of very minor importance. The mode of transmission of the twinning character in dairy cattle is obscured by its relatively rare and incomplete expression, its sex-limited and age-limited appearance, and the small number of offspring inherent in cattle. The factual manifestations of transmission in two cow families over a number of generations provided impressive indications that twinning exhibits Mendelian segregation and seems to be under the control of a small number of genes. The character of twinning should be recessive with gene interactions or modifications. By making use of these findings it is feasible that in practical breeding operations twinning in dairy cattle could be influenced considerably in either direction.

The question then arises as to whether twinning is a desirable character in dairy cattle. If the available data all are accepted at their face value, twinning is associated with the increased production of calves by sheer numbers; if this fact is scrutinized from the actual reproducing value of the female line, the contrary is the case. Of the theoretical twin sex-ratio,  $1 \text{ ♂♂} : 2 \text{ ♂♀} : 1 \text{ ♀♀}$ , for all practical purposes only the female pairs are suitable for reproduction in the direct female lines. Theoretically, the number of these female twins would just be equal to the number of single females. Since twin calves have a higher mortality rate at birth and, apparently, a lower vitality throughout life than single calves, the real fact is that twinning has a harmful effect on the continuity of the female lines.



If the face value of data favoring the association of twinning with longevity is examined closely, the degree of association diminishes considerably. Twin births occur generally late in life, increase with advancing age, and are incomplete in appearance. If every animal in a group is expected to show this character, almost all the animals of this group have undergone an intense selection with respect to age. Therefore, almost any comparison of twinning in regard to age is of very questionable reliability.

Definite disadvantages of twin births include shortened gestation periods, greater parturition difficulties with subsequent increases in retained placentae, decreased conception rate, lower breeding efficiency, and increased sterility.

In summarizing all these factors, there is no doubt that twinning definitely is an undesirable character in dairy cattle, and efforts should be made to reduce its appearance by proper breeding methods and selection.

#### REFERENCES

- (1) BARTLETT, J. W., AND MARGOLIN, S. A Comparison of Inbreeding and Outbreeding Holstein-Friesian Cattle. N. J. Agr. Expt. Sta. Bull. 712. 1944.
- (2) BARTLETT, J. W., REECE, R. P., AND LEPARD, O. L. The Influence of Inbreeding on Birth Weight, Rate of Growth, and Type of Dairy Cattle. J. Animal Sci., 1: 206-212. 1942.
- (3) BARTLETT, J. W., REECE, R. P., AND MIXNER, J. P. Inbreeding and Outbreeding Holstein-Friesian Cattle in an Attempt to Establish Genetic Factors for High Milk Production and High Fat Test. N. J. Agr. Expt. Sta. Bull. 667. 1939.
- (4) ESPE, D. Secretion of Milk. 2nd Ed. The Iowa State College Press, Ames, Iowa. 1946.
- (5) GAINES, W. L. The Energy Basis of Measuring Milk Yield in Dairy Cows. Ill. Agr. Expt. Sta. Bull. 453. 1928.
- (6) HAMMOND, J., JR., AND BHATTACHARYA, P. Control of Ovulation in the Cow. J. Agr. Sci., 34: 1-5. 1944.
- (7) HEWITT, A. C. J. Twinning in Cattle. J. Dairy Research, 5: 101-107. 1937.
- (8) LUSH, J. L. Animal Breeding Plans. 3rd Ed. The Iowa State College Press, Ames, Iowa. 1945.
- (9) LUSH, R. H. Inheritance of Twinning. J. Heredity, 16: 273-279. 1925.
- (10) RICE, V. A. Breeding and Improvement of Farm Animals. 3rd Ed. McGraw-Hill Book Company, Inc., New York, N. Y. 1942.
- (11) WEBER, W. Untersuchungen über die Häufigkeit und Erblichkeit der Disposition zu Zwillingsgeburten beim Simmentalerrind. Arch. Julius Klaus Stiftung, 20: 307-361. 1945.
- (12) WILLIAMS, W. L. Veterinary Obstetrics. 2nd Ed. Published by the Author, Ithaca, N. Y. 1931.

## A STUDY OF THE BROWNING REACTION IN WHOLE MILK POWDER AND ICE CREAM MIX POWDER<sup>1</sup>

N. P. TARASSUK AND E. L. JACK

*Division of Dairy Industry, University of California, Davis*

The development of a brown color during storage is an index of deterioration in many foods. Browning during storage usually is not observed in commercial dry milk, but some observations on milk powders prepared for other purposes led to the consideration that incipient browning might be related to the development of stale and oxidized flavors in milk powder. Consequently, it seemed advisable to investigate the factors contributing to browning and the possible relationship to other deteriorative changes in milk powder.

### REVIEW OF LITERATURE

The literature on the browning reaction in foods is voluminous. Excellent reviews of literature on darkening of various foods and fundamental aspects of the browning reaction have been presented in project reports, Committee on Food Research, QMC, in the last two years. The literature on browning reaction in dairy products has been reviewed recently by Sharp and Stewart (11). The browning in dairy products, similar to other foods, is attributed to two possible reactions: the caramelization of lactose and a Maillard-type reaction between lactose and milk proteins leading to formation of amino-sugar compounds. Webb (12) believes that a lactose-amino combination may account for much of the browning of autoclaved milk, with caramelization of lactose by phosphates as a contributing factor. Regardless of the mechanism of the reaction, the intensity of browning produced in milk is known to be influenced by certain factors, namely, the pH of milk (9, 13), lactose concentration (6, 9, 11), and temperature and time of heating (9, 13). The major chemical change in milk related to the browning reaction is a partial conversion of lactose into acids (7, 14). The acids produced are mainly lactic and formic (3, 7). Kometiani (7) could not account for the total increase in acidity in browning as derived from lactose. He attributed part of the increase in acidity to an increase in free carboxyl groups in the casein molecule.

The published information on browning of dry milk products is limited to the study of Doob *et al.* (2) on browning of dried whey and skim milk. Both products were roll-dried and none of the samples studied was gas-packed. The browning of these products was affected chiefly by moisture

Received for publication November 26, 1947.

<sup>1</sup> The subject matter of this paper has been undertaken in cooperation with the Quartermaster Corps Committee on Food Research.

content, temperature, and time of storage. According to the authors, browning is markedly accelerated at temperatures above 30° C.; however, even at 50° C. browning could be inhibited by low moisture content. An increase in titratable acidity and a decrease in pH accompanied browning of dried whey and skim milk.

#### EXPERIMENTAL PROCEDURE

All powders of whole milk and ice cream mix used in this study were made by the spray process under standard commercial conditions of processing. Ice cream mix powder was prepared in a commercial plant with a Rogers-type drier. Whole milk powder was prepared in a commercial experimental plant equipped with Mojonnier stainless steel processing equipment and Mojonnier drier. All samples were packed and stored in 1-lb. tin cans unless otherwise specified. Gassing of samples refers to evacuation and gassing with nitrogen. Gas analysis was made according to procedure of Peters and Van Slyke (8) with a Haldane gas apparatus. The pressure of gas in cans was taken at 22–24° C. with an attached gas apparatus manometer built for this purpose. The degree of browning (except for the first experiment) was measured by visual comparison of the sample with a set of dry powder standards, the procedure developed by Doob *et al.* (2). These standards are made up of mixtures of potassium chromate, ferric oxide, Norrit and sodium chloride to give colors ranging from white to medium brown in 14 divisions and numbered from 0 for the white to 13 for the darkest standard. Ascorbic acid and total reducing substances were determined by indophenol titration, as described by Sharp (10) and modified by Doan and Josephson (1). In one experiment the reducing substances were determined also by a modified Chapman method and expressed in terms of ferricyanide values. Moisture was determined by the toluol-distillation method, and the solubility index by the method recommended by the American Dry Milk Institute. The QMC score card of 1 to 15 was used in assigning the numerical value of the organoleptic score.

#### EXPERIMENTAL RESULTS

##### *Experiment I. Effect of high humidity on browning of milk powder.*

In this experiment, samples of freshly prepared whole-milk powder were vacuum-packed in special laminated packages, shown by previous experiments to be water-vapor permeable but supposedly not air permeable.<sup>2</sup> The vacuum-packed samples were placed in a wet incubator at 95 per cent relative humidity and the thermostat set at 40° C. During the experiment the thermostat stuck and the temperature rose to 45° C. or more. This

<sup>2</sup>The authors are indebted to Mr. W. C. Cole and Mr. E. S. Chase, Research Laboratory, Arden Farms, Los Angeles, for their valuable contribution in securing the data of Experiments I and II.



increase in temperature undoubtedly accelerated the changes under consideration but did not destroy the value of the experiment. Samples were withdrawn at intervals from the incubator for testing. Results of these tests are presented in table 1.

Storage of whole milk powder under high relative humidity at 40 to 45° C. led to a rapid browning of samples. The browning was accompanied by a decrease in vitamin C, decrease in solubility, and increase in ferricyanide value. With the increase of moisture in powder, there was a development of mild stale flavor at first; but as browning appeared and developed, the stale flavor either decreased or was masked by a burned or caramelized flavor.

*Experiment II. The effect of moisture content of powder on the rate of browning.* The effect of moisture content of powder on the rate of browning also is brought out by the data of Experiment II. In this experiment, samples were prepared to contain approximately 4 per cent and 7 per cent moisture by adding water drop by drop from a pipette to milk powder as it was stirred in a Hamilton Beach Mixer. The prepared samples were packed: (a) in air, using pint mayonnaise jars, and (b) vacuum-packed (vacuum of 28 inches) in special vacuum-holding laminated packages. Samples were stored in an incubator at 45° C. Subsequent analyses showed that within the same moisture group of samples, there was a variation in the moisture up to 0.9 per cent. The vacuum-packed samples lost some moisture as the result of subjecting the samples to vacuum treatment. The data on browning and reducing groups (ferricyanide value) of the samples are presented in table 2. At 4 per cent moisture and storage at 45° C. there was no significant change in color on storage for 26 days. Samples containing 7 per cent moisture darkened significantly within the first 2 weeks of storage. Air-packed powder showed no consistent and significant difference in the degree of browning as compared with the same powder vacuum-packed at the levels of moisture tested.

The solubility index was run on the samples after 2 and 3 days of incubation. By then, the high-moisture samples were very insoluble, and continuation of the tests seemed unnecessary.

*Experiment III. Gas changes during browning of whole milk powder.* Early observations on browning of some ice cream mix powder samples packed under air and under 3 per cent of oxygen indicated that the browning was retarded in gas-packed samples. In one case, ungasged ice cream mix powder on storage at 45° C. for 3 months was dark brown and the same product gassed was only very slightly discolored. These samples originally were below 2 per cent in moisture but were badly contaminated with iron, which might explain why browning took place at that level of moisture in powder. Other samples of powder, free from iron contamination and with

moisture content of 2.5 per cent or lower, did not show any discoloration on storage at 44–45° C. for over 1 year. It is probable that iron accelerates browning in milk powder, as it has been shown to do in orange juice (5) and in lemonade and orangeade powders (4). This acceleration of browning does not explain the difference, however, in degree of browning in gassed and ungassed samples, unless removal of oxygen inhibits browning as in the case of orange juice (5). The data of Experiment II in this report show clearly that in samples of powder of high moisture content the degree of browning was not affected significantly by a partial removal of oxygen.

In Experiment III the samples of whole-milk powder were prepared to contain approximately 7, 4, and 2 per cent of moisture. This was accomplished by placing freshly prepared powder at 2 per cent moisture in a special stainless steel chamber of high relative humidity. The powder in this chamber was mixed frequently, and incorporation of moisture up to 7 per cent was accomplished in 44 hours. The samples at each level of moisture content were packed under three levels of oxygen, that of 21 per cent, about 10 per cent, and less than 2 per cent by packing, respectively, in air, with single gassing and double gassing. All samples were packed to contain 14 oz. of powder. The free-space gas volume, as calculated from gas pressures at 23° C., was  $495 \pm 10$  ml.

All samples were stored at 40° C. After 5 months of storage, the samples of 2 per cent and 4 per cent moisture failed to show a significant discoloration at 40° C. and were placed in the incubator at 60° C. for further storage. The data on browning, uptake of oxygen, production of carbon dioxide, and changes in moisture and flavor are presented in tables 3, 4, and 5 for powders of 7, 4 and 2 per cent, numbered as series 29II, 29I and 29, respectively. The partial pressure values for carbon dioxide and oxygen were calculated by converting per cent of gas on wet basis to per cent on a dry basis and multiplying this figure by total pressure on a dry basis.

Figures 1 and 2 show the rate of browning and gas changes during browning of high-moisture powder. The relationship between degree of browning and carbon dioxide production in powder of 2 per cent moisture packed under various levels of oxygen is shown in figure 3.

The data on browning and other changes of ice cream mix powder stored at 20, 37 and 45° C. are given in table 6. This powder had no added sugar and contained 53.55 per cent fat. The powder had 0.04 per cent tannic acid added as an antioxidant during the processing of the mix.

#### DISCUSSION

The conditions under which dry milk and ice cream mix powder undergo darkening or browning as a result of aging are apparent from the data presented. The browning of powder is a function of its moisture content

TABLE 3  
*Browning of whole milk powder of 7% moisture packed under various levels of oxygen (storage at 40° C.)*

Oxygen level	Sample	Storage time (days)	Moisture (%)	Color	Partial pressure		Flavor
					CO <sub>2</sub>	O <sub>2</sub>	
21	1	0	7.00	0.9	5.0	146.4	Good
	2	6	7.04	1.5	21.3	103.4	Burnt & stale
	3	28	7.00	2.6	39.7	1.3	Burnt & stale
	4	47	7.00	4.0	49.4	0.3	Caramelized & stale
	5	70	6.90	4.7	61.2	0.3	Caramelized & stale
	6	104	7.36	6.7			
10	1	0	7.22	0.9	3.5	59.0	Good
	2	6	7.34	1.7	11.1	32.4	Burnt & stale
	3	28	7.20	3.8	28.7	1.4	Caramelized & stale
	4	47	7.40	4.3			Caramelized & stale
	5	70	7.52	6.9	48.5	0.2	Caramelized & stale
	6	104	7.30	7.1	53.6	0.2	Caramelized & stale
2	1	0	6.74	1.0	1.1	2.8	Good
	2	6	6.70	1.8	6.1	1.0	Burnt
	3	28	7.00	5.3	22.7	0.8	Caramelized & v. sl. stale
	4	47	6.80	4.8			Caramelized & v. sl. stale
	5	70	7.14	7.5	42.7	0.7	Caramelized & v. sl. stale
	6	104	7.20	8.0	54.7	0.2	Caramelized & v. sl. stale



TABLE 4  
*Browning of whole milk powder of 4% moisture packed under various levels of oxygen*

Powder	Sample no.	Storage time (days)	Storage temperature (° C.)	Moisture (%)	Color	Partial pressure		Flavor
						CO <sub>2</sub>	O <sub>2</sub>	
29I	1	0	....	3.93	0.7	(mm.) 2.0	(mm.) 156.8	Good
	2	19	40	3.73	0.9	4.7	142.8	.....
	3	61	40	3.62	1.0	13.2	84.6	.....
	4	152	40	.....	1.5	.....	<1.0	Stale
	5	11	60	5.25	<13.0	215.0	.....	V. caramelized
	6	15	60	5.20	>13.0	>230.0	.....	V. caramelized
29IA	1	0	....	3.70	0.7	1.5	91.5	Good
	2	19	40	4.04	0.9	3.2	86.5	.....
	3	61	40	4.00	1.0	13.1	35.7	.....
	4	152	40	.....	1.5	.....	<1.0	Stale
	5	11	60	5.56	<13.0	277.0	.....	V. caramelized
	6	17	60	5.56	>13.0	>250.0	.....	V. caramelized
29LAB	1	0	....	3.87	0.7	1.1	6.6	Good
	2	19	40	3.96	0.9	2.3	5.9	.....
	3	61	40	3.80	0.9	3.6	2.5	.....
	4	152	40	.....	1.5	.....	.....	Sl. stale
	5	11	60	4.84	<13.0	119.0	<1.0	V. caramelized
	6	17	60	4.80	>13.0	>260.0	0.0	V. caramelized

TABLE 5  
*Browning of whole milk powder of 2% moisture packed under various levels of oxygen*

Powder	Sample no.	Storage time (days)	Storage temperature (° C.)	Moisture (%)	Color	Partial pressure		Flavor
						CO <sub>2</sub>	O <sub>2</sub>	
29	1	0	.....	1.95	0.6	2.8	141.7	Good
	2	152	40	.....	0.6	.....	.....	.....
	3	17	60	2.30	1.5	16.6	<1.0	Stale
	4	43	60	2.00	1.8	23.4	<1.0	Stale
	5	108	60	2.06	4.2	80.2	<1.0	Stale & caramelized
	6	157	60	2.20	4.5	74.0	<1.0	Stale & caramelized
29A	1	0	.....	1.82	0.6	2.1	88.2	Good
	2	152	40	.....	0.6	.....	.....	.....
	3	17	60	1.70	1.5	16.2	<1.0	Stale
	4	43	60	1.80	1.8	20.8	<1.0	Stale
	5	85	60	2.10	4.5	72.6	<1.0	Stale & caramelized
	6	108	60	2.40	3.8	55.2	<1.0	Stale & caramelized
	7	157	60	2.90	7.2	123.0	<1.0	Sl. stale & caramelized
29AB	1	0	.....	1.72	0.6	1.6	3.5	Good
	2	152	40	.....	0.6	.....	.....	.....
	3	17	60	1.40	1.5	11.5	<1.0	Good
	4	43	60	2.00	1.8	15.1	<1.0	Good
	5	85	60	1.34	2.0	21.1	.....	.....
	6	108	60	1.80	2.5	27.7	.....	.....
	7	157	60	2.32	3.5	42.4	.....	Sl. caramelized

TABLE 6  
Browning of ice cream mix powder

Sample no.	Packaging	Storage temperature (° C.)	Storage time (days)	Moisture (%)	Color	CO <sub>2</sub> (%)	O <sub>2</sub> (%)	Titratable acidity (ml. 0.1 N NaOH)	Flavor
1	No gassing	20	26	1.5	1.5	0.12	20.7	1.36	Good
2	No gassing	20	80	.....	1.5	0.18	19.77	.....	.....
3	No gassing	20	431	1.60	1.5	0.50	13.70	1.36	Stale
4	Gassed	20	26	1.50	1.5	0.03	2.28	.....	Good
5	Gassed	20	80	.....	1.5	0.08	1.49	.....	.....
6	No gassing	37	0	1.5	1.5	0.12	20.70	1.36	Good
7	No gassing	37	416	1.96	1.5	1.49	2.40	1.40	V. stale
8	Gassed	37	0	1.50	1.5	0.03	2.28	1.36	Good
9	Gassed	37	416	1.60	1.7	0.31	0.03	1.36	V. sl. stale
10	No gassing	45	0	1.50	1.5	0.12	20.70	1.36	Good
11	No gassing	45	14	1.60	1.5	0.39	19.02	.....	.....
12	No gassing	45	55	.....	1.5	0.91	13.49	.....	.....
13	No gassing	45	416	1.95	4.0	.....	.....	2.54	Oxidized & caramelized
14	Gassed	45	0	1.50	1.5	0.03	2.28	1.36	Good
15	Gassed	45	55	.....	1.5	0.44	0.84	.....	.....
16	Gassed	45	416	1.70	2.7	1.28	0.06	2.20	Sl. stale, sl. caramelized
17 <sup>a</sup>	No gassing	60	40	2.20	10.0	.....	.....	5.10	Caramelized & stale
18 <sup>a</sup>	Gassed	60	40	1.90	7.0	.....	.....	4.60	Caramelized

<sup>a</sup> Samples 17 and 18 are duplicates of Samples 7 and 9, respectively. They were stored at 37° C. for 416 days and then placed in an incubator at 60° C.



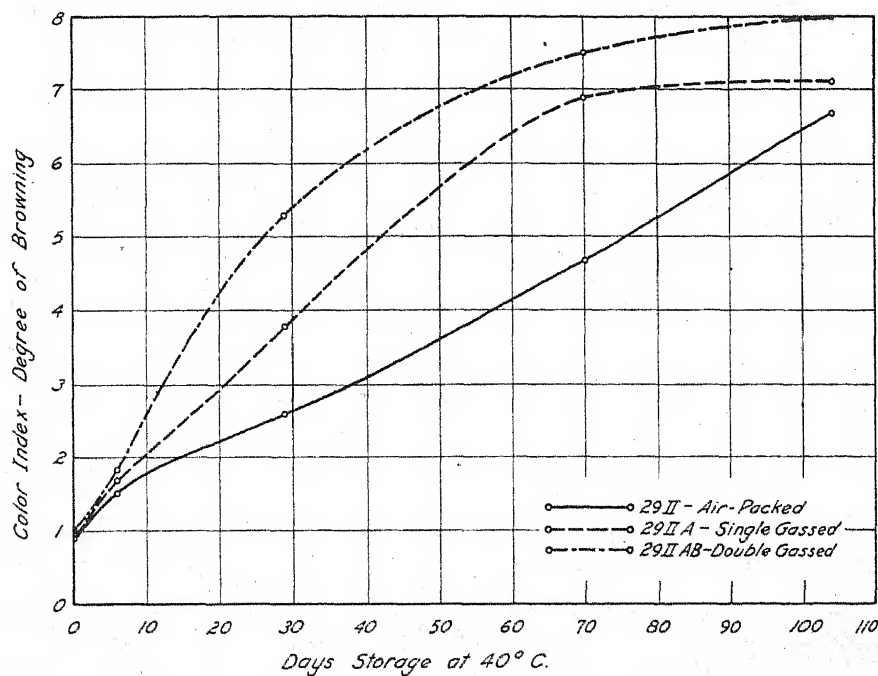


FIG. 1. Rate of browning of whole milk powder of 7% moisture (Series 29II).

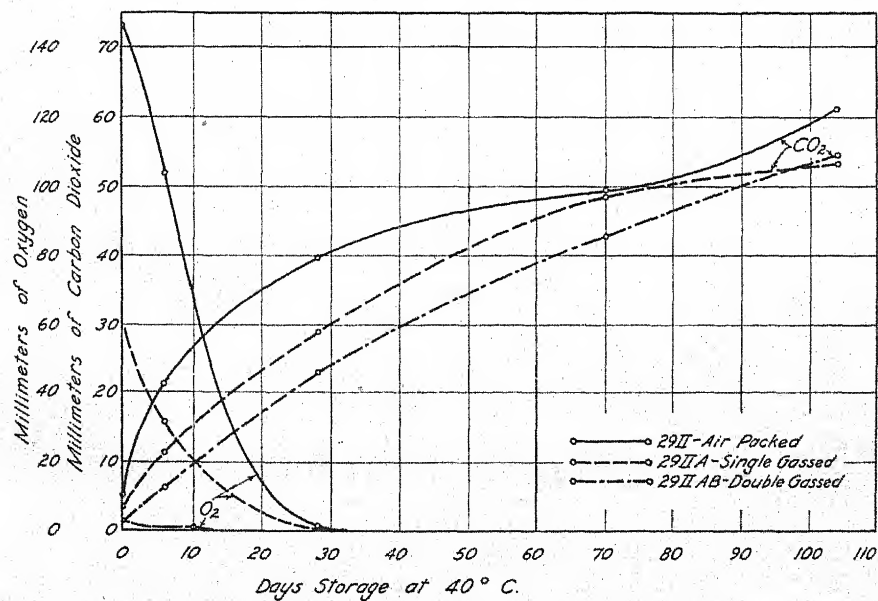


FIG. 2. Gas changes during browning of whole milk powder of 7% moisture (Series 29II).

and temperature of storage. Other factors, such as contamination with iron and possibly copper, certain added polyphenol compounds as antioxidants, and possibly vanillin in ice cream mix powder, may accelerate the rate of browning, but these factors are of comparatively minor importance in their relation to the browning of powder.

In general, the browning of powder is accompanied by: (a) production of carbon dioxide, (b) uptake of oxygen, (c) increase in reducing groups, (d) very marked decrease in solubility, (e) increase in titratable acidity,

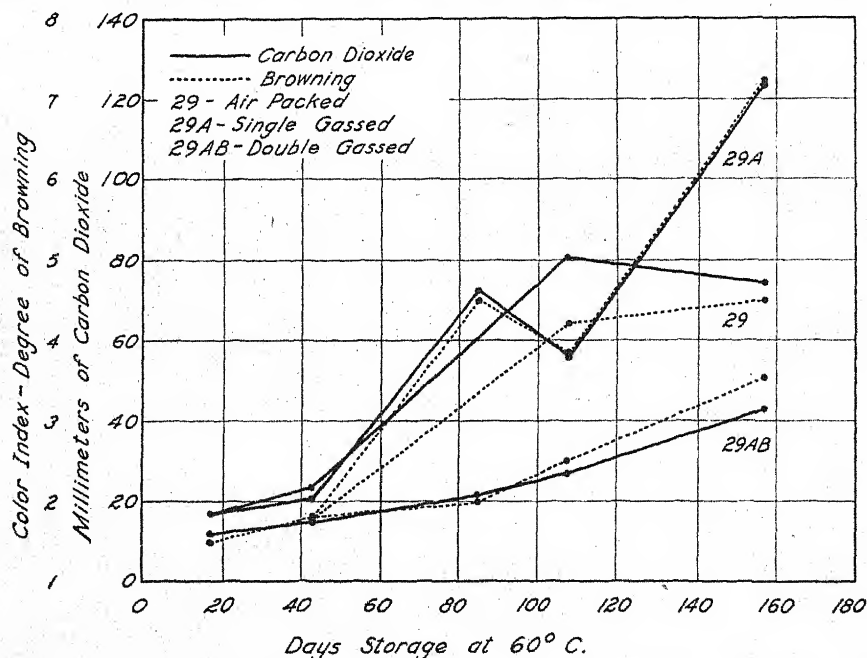


FIG. 3. Relationship between degree of browning and carbon dioxide production in whole milk powder of 2% moisture (Series 29).

and (f) development of caramelized flavor. In the advanced stages of browning there is also an increase in moisture content of powder. There was little, if any, increase in moisture in powder of 7 per cent moisture as compared to the same powder of lower moisture content.

In powder of 7 per cent moisture stored at 40° C., noticeable discoloration of powder takes place within a few days. On storage for a month, the powder becomes distinctly brown, with the appearance of caramelized flavor. At this time the oxygen practically is all gone and the partial pressure of carbon dioxide is increased to about 30 mm. under the conditions described. After the oxygen is gone, the browning and production of carbon dioxide continue but at slower rates.

In powders of 4 per cent and 7 per cent moisture content, storage at a

partial pressure of oxygen below 7 mm. (less than 1 per cent) did not retard the rate of browning. In fact, the samples packed under less than 2 per cent of oxygen have shown greater darkening than air-packed samples (see table 3 and fig. 1).

The browning of powder of 2 per cent moisture or less seemingly was retarded by packing the powder at the level of about 2 per cent of oxygen (see fig. 3 and table 6). It is possible that the retarding effect was due, at least partially, to a lower moisture content of gas-packed samples resulting from the vacuum treatment in the process of gassing.

It is evident from the data of tables 1, 4, 5 and 6 that both dry milk and ice cream mix powder of a moisture content below 4 per cent and stored at 40° C. or lower do not darken or brown in storage. Other samples of dry milk and ice cream mix powder of less than 3 per cent moisture have been stored at 40 and at 30° C. for over 2 years without showing any noticeable discoloration.

The usual deterioration in flavor of dry milk and ice cream mix powder in storage is independent of browning. Under the conditions of high available oxygen, as in the case of air-packed powder and storage at 40° C., the stale and oxidized flavor will develop with no browning at all. The same is true for a storage of powder at room temperature for a long period of time. The caramelized flavor is the only flavor that is produced by browning, and its intensity parallels the degree of browning. Caramelized flavor in gassed samples is a typical flavor of caramel. The development of stale or oxidized flavor apparently ceases when browning begins. There is no evidence that stale or oxidized flavors which have developed prior to browning disappear as browning progresses. These flavors merely are reduced or covered up when caramelized flavor appears.

#### SUMMARY

The conditions with respect to moisture content of powder, temperature of storage, and level of oxygen in gas-free space of container, as they may affect the browning of dry milk and ice cream mix powder as a result of aging, are given. The changes accompanying the browning, such as production of carbon dioxide, uptake of oxygen, increase in reducing groups, decrease in solubility and development of caramelized flavor, have been studied, and the extent of these changes in relation to the degree of browning is presented.

The darkening or browning of dry milk and ice cream mix powder, unlike some other dehydrated foods, is not related to the usual storage deterioration in flavor. The most common defective storage flavors of dry milk are the stale and the oxidized flavors. The development of these flavors in milk and ice cream mix powder is not the result of incipient browning.



In fact, it appears that the products of browning reaction inhibit the development of these flavors. Browning is accompanied by a development of a specific flavor, a caramelized flavor.

The browning does not take place in dry milk or ice cream mix powder when the above products are stored at 40° C. or lower if their moisture content is below 4 per cent.

#### REFERENCES

- (1) DOAN, F. J., AND JOSEPHSON, D. V. Observations on the Ascorbic Acid Content of Evaporated Milk. *J. Dairy Sci.*, 26: 1031-1041. 1943.
- (2) DOOB, H., WILLMAN, F., AND SHARP, P. F. The Influence of Moisture on Browning of Dried Whey and Skim Milk. *Ind. Eng. Chem.*, 34: 1460. 1942.
- (3) GOULD, I. A. The Formation of Volatile Acids in Milk by High Temperature Heat Treatment. *J. Dairy Sci.*, 28: 379-386. 1945.
- (4) ISAAC, W. E. Storage of Synthetic Citrus Powders. *Ind. Eng. Chem.*, 35: 470. 1943.
- (5) JOSLYN, M. A., AND MARSH, G. L. Browning of Orange Juice. *Ind. Eng. Chem.*, 27: 186-189. 1935.
- (6) KASS, J. P., AND PALMER, L. S. Browning of Autoclaved Milk. *Ind. Eng. Chem.*, 32: 1360-1366. 1940.
- (7) KOMETIANI, P. A. Veranderungen einiger Milchbestandteile durch Erhitzen. *Milchw. Forsch.*, 12: 433-454. 1931.
- (8) PETERS, J. P., AND VAN SLYKE, D. D. Quantitative Clinical Chemistry. Vol. 2. Williams and Wilkins, Baltimore, Md. 1931.
- (9) RAMSEY, R. J., TRACY, P. H., AND RUEHE, H. A. The Use of Corn Sugar in the Manufacture of Sweetened Condensed Skim Milk. *J. Dairy Sci.*, 16: 17-31. 1933.
- (10) SHARP, P. F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. *J. Dairy Sci.*, 21: 85-88. 1938.
- (11) SHARP, P. F., AND STEWART, A. P. Terminal Report. Project Report, Committee on Food Research, Q.M.C. 1946.
- (12) WEBB, B. H. Color Development in Lactose Solutions during Heating with Special Reference to the Color of Evaporated Milk. *J. Dairy Sci.*, 18: 81-96. 1935.
- (13) WEBB, B. H., AND HOLM, G. E. Color of Evaporated Milks. *J. Dairy Sci.*, 13: 25-39. 1930.
- (14) WHITTIER, E. O., AND BENTON, A. The Formation of Acid in Milk by Heating. *J. Dairy Sci.*, 10: 126-138. 1927.

## IRON AND COPPER CONTENT OF NON-MILK PRODUCTS COMMONLY USED IN ICE CREAM

HARRY PYENSON AND P. H. TRACY

*Department of Food Technology, University of Illinois, Urbana*

There is a lack of information on the iron and copper content of the non-dairy ingredients that commonly are used in conjunction with dairy products in the manufacture of ice cream. These materials include such products as stabilizers, emulsifying agents, sugar, cocoas, vanillas, chocolate liquors and coatings, and flavoring extracts. It is possible that one or more of these ingredients may contain enough copper or iron to accelerate the development of off-flavors of the oxidative type. Such metal contamination would be particularly important in the manufacture of dried ice cream mix that may be stored 6 to 12 months before using.

It was thought advisable, therefore, to study the iron and copper content of the previously mentioned materials. Accordingly, 74 samples of commercial non-dairy products commonly used in ice cream were analyzed. The iron content was determined by the method of Pyenson and Tracy (5) and the copper analyses were made by the method of Hetrick and Tracy (4).

### EXPERIMENTAL RESULTS

*The iron and copper content of stabilizers and sugars.* Eighteen samples of stabilizers, gums, emulsifying agents, and sugars were analyzed for iron and copper. Magnesium nitrate (1) was added to Kragel,<sup>1</sup> sodium alginate, Irish moss, Vestirine and Gelox after carbonization to aid in the ashing. Two milliliters of a saturated solution of magnesium nitrate added after carbonization was found satisfactory to give a white, soluble ash. The results of the analyses are given in table 1.

All stabilizers except Gelox contained considerable amounts of iron. Irish moss contained 0.219 per cent of iron, which would be considered more than a trace amount. The emulsifying agents contained from 1 to 59 p.p.m. of iron. Egg yolk, which is sometimes used in ice cream as an emulsifying agent, contained 59 p.p.m. of iron. The sugars analyzed contained only small amounts of iron.

The copper content of the stabilizers varied from 0.92 to 10.0 p.p.m. Irish moss, locust bean gum, Kragel, and sodium alginate contained the most copper of the ten stabilizers analyzed. Glycerol monostearate did not contain any copper and Mixacoid contained practically none. Na-Pe-Co and egg yolk contained 2.85 and 3.35 p.p.m., respectively, of copper. Su-

Received for publication December 3, 1947.

<sup>1</sup> Now modified and known as Kragelene.

crose, dextrose, and enzyme-converted corn sirup were found to contain less than 1 p.p.m.

*The iron and copper content of American and Dutch process cocoas.* The iron and copper contents of 17 samples of cocoa from five different manufacturers were determined (table 2). Of these samples, 13 had been treated with alkali (Dutch process) and four were untreated (American process).

The iron values for the American process cocoas varied from 73 to 119 p.p.m., with an average for the four cocoas of 92 p.p.m. In the Dutch

TABLE 1

*The iron and copper content of some stabilizers, emulsifying agents and sugars*

Sample no.		Iron (p.p.m.)	Copper (p.p.m.)
	Stabilizers		
1	Gelatin (275 Bloom-pigskin)	14.8	2.21
2	Gelatin (125 Bloom-calfskin)	9.0	2.56
3	Gelox	1.2	2.0
4	Vestirine	28.0	1.75
5	Kragel	61.0	9.0
6	Sodium alginate	97.0	6.25
7	Irish moss	2190.0	10.0
8	Locust bean gum	16.4	9.0
9	Gum oat	47.6	5.4
10	Karaya gum	26.4	0.92
	Emulsifying agents		
11	Na-Pe-Co	19.4	2.85
12	Mixacoid	1.0	0.25
13	Glycerol monostearate	2.0	0.00
14	Egg yolk	59.0	3.35
	Sugars		
15	Sucrose, lot no. 1	0.9	0.15
16	Sucrose, lot no. 2	1.2	0.20
17	Dextrose	0.4	0.40
18	Enzyme-converted corn sirup	1.1	0.70

process cocoas the iron content averaged 117 p.p.m., with only three of them having an iron content under 100 p.p.m. Eight of the samples had an iron content between 110 and 149 p.p.m. There is some evidence that Dutch process cocoa contains more iron than American process cocoa, especially when comparisons are made between the two cocoas from the same manufacturer.

Dahlberg (2) found that a greenish-black discoloration of chocolate ice cream was caused by ferric tannate and that the cocoas that had a slightly alkaline pH value (Dutch process) were the only ones that produced the defect. The results of the present study suggest that the greater iron content of the Dutch processed product also may be a factor.



TABLE 2

*The iron and copper content of American and Dutch process cocoas*

Sample no.	Process	Iron (p.p.m.)	Copper (p.p.m.)
	Brand A		
1	Dutch	94.5	20.6
2	American	73.0	21.0
3	Dutch	136.7	21.0
4	Dutch	142.7	21.2
5	American	84.7	20.6
6	Dutch	134.0	20.6
7	Dutch	134.0	21.0
	Brand B		
8	Dutch	149.0	22.2
9	American	119.0	23.4
10	Dutch	120.5	24.0
11	Dutch	141.0	21.6
	Brand C		
12	American	92.0	22.4
13	Dutch	110.0	23.5
14	Dutch	108.0	23.6
15	Dutch	105.0	31.2
	Brand D		
16	Dutch	69.0	27.3
	Brand E		
17	Dutch	75.0	28.3

The copper content of the samples varied from 20.6 to 31.2 p.p.m. American and Dutch process cocoas gave about the same copper values. The copper content of cocoas was more uniform than the iron content.

*The iron and copper content of chocolate liquors and coating.* Seven samples of chocolate liquor and one sample of milk coating were analyzed for copper and iron. The results are given in table 3.

Like cocoas, chocolate liquors and coating contain considerable quantities of iron and copper. The iron content of chocolate liquors and coating varied from 14 to 105 p.p.m., and the copper varied from 2.3 to 27 p.p.m.

TABLE 3

*The iron and copper content of chocolate liquor and coating*

Sample no.	Brand	Iron (p.p.m.)	Copper (p.p.m.)
1	A	100.8	20.0
2	A (Milk coating)	14.0	2.3
3	B	59.0	14.9
4	B	71.0	15.5
5	C	104.5	27.0
6	D	103.0	24.8
7	E	105.0	18.9
8	F	19.4	9.8

*The iron and copper content of flavoring.* Twenty-two vanillas obtained from nine manufacturers were analyzed for iron and copper. The results are listed in table 4.

The three samples of powdered vanillas were uniformly low in iron and contained less than 2 p.p.m. copper. Powdered vanillas are made by grinding vanilla beans and combining the ground beans with a carrier like

TABLE 4  
*The iron and copper content of vanillas*

Sample no.	Brand	Iron (p.p.m.)	Copper (p.p.m.)
Powdered vanillas			
1	A	4.8	1.35
2	A	4.0	1.90
3	B	1.4	0.45
Concentrated vanillas			
4	C	59.0	9.45
5	C	58.4	4.95
6	C	43.8	36.5
7	C	32.6	4.3
8	D	2.0	9.5
9	D	4.0	10.5
Vanilla extracts			
10	E	4.4	15.5
11	A	33.6	19.5
12	D	0.8	4.9
13	B	1.5	2.2
14	E	0.6	2.7
15	F	7.8	3.75
16	E	1.9	9.30
Imitation or partially imitation vanillas			
17	C	58.0	3.2
18	B	46.0	7.75
19	F	4.4	3.7
20	E	5.3	3.25
21	E	3.0	1.2
22	G	4.4	15.5

sucrose or glucose. The vanilla bean as it exists in nature appears to be relatively low in iron and copper.

The concentrated vanillas studied were found to have a rather high iron and copper content. The iron content of single strength vanilla extracts varied from 0.6 to 33.6 p.p.m. Five out of the seven samples had an iron content under 4.4 p.p.m. The copper content also varied considerably; the minimum was 2.2 p.p.m. and the maximum 19.5 p.p.m.

The iron content of the imitation or partially imitation vanillas varied from 3 to 58 p.p.m. and the copper content from 1.2 to 15.5 p.p.m. Represented in this lot were six samples from five different manufacturers.

The iron and copper content of fruit flavors and extracts. Most of the samples of fruit flavors and extracts analyzed (table 5) contained less than 5 p.p.m. of iron. Six out of the nine samples contained less than 5 p.p.m. of copper. Lime flavor, black raspberry concentrate and strawberry flavor were comparatively high in copper content.

TABLE 5

*The iron and copper content of some fruit flavors and extracts*

Sample no.		Iron	Copper
		(p.p.m.)	(p.p.m.)
	Brand A		
1	Orange emulsion conc.	3.6	0.25
2	Lemon emulsion conc.	3.4	0.25
	Brand B		
3	Black raspberry natural flavor conc.	18.6	6.9
4	Imitation pineapple flavor	3.9	0.45
5	Strawberry flavor	5.6	5.05
6	Peach flavor conc.	1.3	2.25
7	Lime flavor	1.2	11.25
8	Pistachio imitation conc.	0.5	4.05
9	Imitation banana	1.0	0.65

## DISCUSSION

The determination of copper by the direct carbamate method used in this study does not entirely eliminate the interference of nickel. Nickel exhibits maximum absorption at a wave length of 385 m $\mu$ , while copper exhibits maximum absorption at a wave length of 440 m $\mu$ . Hetrick and Tracy (4) state that when 5  $\gamma$  of nickel are added to 5  $\gamma$  of copper, the error is +0.6  $\gamma$ . Studies in the wave length at which maximum absorption occurs of the materials reported in this paper indicate that there was little, if any, nickel present.

The non-dairy products individually would produce only insignificant increases in the iron and copper content of ice cream. The Irish moss sample studied would be an exception, as it contained over 0.2 per cent iron. While the copper and iron content of the milk-product ingredients ordinarily would have a major bearing on the iron and copper content of the finished ice cream, the total added by non-milk products could be of such quantity as to be an important factor in the development of oxidized flavors.

Observations by Dahle and Folkers (3) and Tracy *et al.* (7) have shown that ice creams containing small amounts of fruit such as strawberries and pineapple develop a stale and/or oxidized flavor sooner than does vanilla ice cream. These authors believe that the off-flavor is due to the presence of copper and the acid of the fruit. Dahle and Folkers (3) state



that if the amount of copper in the mix equaled 1.3 p.p.m., the off-flavor always developed. Tracy *et al.* (7) state that in order to prevent the development of a stale metallic flavor in strawberry ice cream, the elimination of copper contamination is necessary. Other fruits found to accelerate the reaction responsible for the off-flavor were oranges and lemons. The copper content of any of the non-dairy products studied conceivably could be a factor in accelerating the development of the oxidized flavor, especially if used in strawberry, pineapple, orange and lemon ice creams.

The vanilla sample no. 4 in table 4 has been shown to have anti-oxygenic properties (6) although it contains relatively large amounts of iron and copper, indicating that a substance may be relatively high in iron and copper and still have antioxygenic properties.

#### CONCLUSIONS

Stabilizers, cocoas, chocolate liquors, sugars, vanillas and fruit extracts were found to contain iron and copper. The copper and iron present in some of these products is thought to be significant from the standpoint of possible cumulative effect in hastening fat oxidation and the development of off-flavor in ice cream.

#### REFERENCES

- (1) COMRIE, A. A. D. The Determination of Copper in Foods. *Analyst*, 60: 532-533. 1935.
- (2) DAHLBERG, A. C. A Greenish-black Discoloration of Chocolate Ice Cream. *J. Dairy Sci.*, 6(5): 455-460. 1923.
- (3) DAHLE, C. D., AND FOLKERS, E. C. Factors Contributing to an Off-flavor in Ice Cream. *J. Dairy Sci.*, 16(6): 529-548. 1933.
- (4) HETRICK, J. H., AND TRACY, P. H. The Determination of Copper in Milk Powder by a Direct Carbamate Method. *J. Milk Technol.*, 8: 1-8. 1945.
- (5) PYENSON, H., AND TRACY, P. H. A 1,10-Phenanthroline Method for the Determination of Iron in Powdered Milk. *J. Dairy Sci.*, 28(5): 401-412. 1945.
- (6) PYENSON, H., AND TRACY, P. H. Manufacture of Powdered Cream for Whipping by Aeration. Unpublished data. Department of Food Technology, Univ. of Illinois. 1945-1947.
- (7) TRACY, P. H., RAMSEY, R. J., AND RUEHE, H. A. A Study of the Causes of a Stale Metallic Flavor in Strawberry Ice Cream. *Ill. Agr. Expt. Sta. Bull.* 407. 1934.

# THE DEVELOPMENT OF FLAVOR IN AMERICAN CHEDDAR CHEESE MADE FROM PASTEURIZED MILK WITH *STREPTOCOCCUS FAECALIS* STARTER<sup>1</sup>

A. C. DAHLBERG AND F. V. KOSIKOWSKY

Department of Dairy Industry, Cornell University,  
Ithaca, New York

This report is the first of several papers dealing with the development of a higher flavor in pasteurized-milk American Cheddar cheese. Cheddar cheese is made by a process that gives a long period for controlled lactic acid fermentation during manufacture. Most investigators have found that commercial lactic starters, chiefly *Streptococcus lactis* and *Streptococcus cremoris*, affect acidity without otherwise greatly affecting curing. As these starters in active growing condition are very important in both cheese manufacture and curing, it is obvious that proper acidity is very important in curing cheese. With the exception of some inoculations of certain lactobacilli, there have been no bacteria found that have aided in the development of good Cheddar cheese flavor. Furthermore, added enzymes, particularly lipases and proteinases, have not given very promising results.

In 1941 Wilson *et al.* (8) compared curing temperatures of 40, 50, and 60° F. They found 40° F. to be best for cheese made from poor milk, but 50° F. was preferred for cheese made from pasteurized milk of good quality. The type of curing was rather uncertain at 60° F. About this same time Dahlberg and Marquardt (2) showed that cheese made from either raw or pasteurized milk of excellent sanitary quality and ripened in vacuum in tin cans uniformly failed to develop Cheddar flavor in a year at 40° F., whereas some Cheddar flavor developed in 4 months at 50° F. and in 2 months at 60° F. In this study cheese made from raw or pasteurized milk of low bacterial count developed flavor uniformly; hence, it is evident that the effect of pasteurization of milk in slowing the curing of cheese is due chiefly to destruction of bacteria rather than the milk enzymes, and that this effect may be overcome in part by higher curing temperatures. The evidence indicates that thermoduric bacteria are a factor in cheese curing or that the pasteurized milk was recontaminated.

Consideration of the problem indicated little chance of success by the usual procedure of isolating bacteria from cheese and using them in

Received for publication December 10, 1947.

<sup>1</sup>This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Lois Phelps and Miss Catherine Verwoert for making many of the chemical analyses, and to Dr. I. C. Gunsalus for determining the decarboxylation activity of the special strain of *S. faecalis* used in this study.

its manufacture, as so much of this work already has been done. Rather, one might consider the characteristics of the bacterium desired in cheese making and curing and then ascertain if such a bacterium exists. For example, the desired bacterium should be universal in milk, as all good milk can be made into good cheese without adding this culture. Probably the organism should survive pasteurization. It should produce lactic acid from lactose rapidly and also be able to use lactates or other compounds in cheese as a source of energy; grow well at temperatures of 50° F. or less, and at temperatures as high as 106° F.; not produce gas in large amounts, although it might need to produce some carbon dioxide, as Dorn and Dahlberg (3) have shown cheese made from excellent milk yielded limited amounts of almost pure carbon dioxide during curing; be non-proteolytic and not produce objectionable flavors and odors. It should grow anaerobically at a pH of 5.0 to 5.5 and at salt concentrations up to 6 per cent, as this concentration is about the maximum that normally occurs in the water in Cheddar cheese. The bacterium which has been described obviously may be *Streptococcus faecalis*, as this organism has the characteristics given. This organism would develop in the cheese milk and in the cheese during curing, especially if stored at 50 or 60° F. White and Sherman (6) recently have found enterococci in all raw and pasteurized milk samples which they tested.

#### EXPERIMENTAL METHODS

A dozen or more cultures of *S. faecalis* were obtained from several laboratories and all of them produced acid too slowly in milk to appear to be promising. The idea remained dormant for a few years and then a search was made in nature for a strain which ferments lactose rapidly, as *S. faecalis* often loses this characteristic when propagated in media which do not contain lactose. Some 15 human adults saved stools from which enterococci were isolated on the penicillin-azide agar of White and Sherman (6), and *S. faecalis* was identified by the characteristics given by Sherman (5). *S. faecalis* is the predominating streptococcus in the digestive tract of man. Approximately 40 or 50 strains of enterococci were isolated before one was found that curdled milk rapidly. A 1 per cent inoculation of this strain incubated at 85° F. curdled milk in less than 18 hours, producing a smooth curd without gas. The flavor of the starter was characteristic and definite, but quite different from ordinary lactic starter. The odor was flat without being objectionable. It was identified as *S. faecalis* and possessed all typical characteristics. It did not ferment glycerol. The tyrosine decarboxylation activity of *S. faecalis* isolated from the starter was  $Q_{CO_2} = 50$  and that isolated from the ripened cheese made with the starter was  $Q_{CO_2} = 60$ . This indicates a moderately active strain for conversion of tyrosine into tyramine.



This *S. faecalis* strain has been carried in milk pasteurized at 200° F. for 1 hour and incubated at 88° F. The curdled starters are held at 40° F. and transferred twice weekly. The starter appears to be pure *S. faecalis* on plating, but no endeavor was made to carry it in sterile milk or other media to assure no loss in acid-producing ability while the research was in progress. Should the starter become contaminated, the *S. faecalis* bacteria could be reisolated and developed as a new starter of the same organism. Pure cultures of this organism have been prepared and are in storage.

The milk used for cheesemaking was a good quality of market milk pasteurized at 143–145° F. for 30 minutes. After cooling to 86° F., the milk was divided into three lots of 300 lb. each. To the first batch of milk was added 2 per cent of Hansen's commercial lactic acid starter; to the second batch, 1 per cent of Hansen's starter and 1 per cent of *S. faecalis* starter; and to the third batch, 2 per cent of *S. faecalis* starter. The milk then was made into cheese according to the time schedule of Wilson (7), using the 4.5-hour schedule from adding rennet to milling the curd, except that no time was allowed for the starters to develop before adding rennet. Acid development was followed by titratable acidity and pH, using a Beckman pH meter, laboratory model G, with glass electrode. After manufacture and pressing, the cheese was vacuum packed in cans and ripened at 50 and 60° F. A few samples were made into 10- or 30-lb. cheese and paraffined in accordance with the usual commercial practice.

The cheese was analyzed for moisture, salt and fat. On the day it was taken from the press, analyses were made for pH, volatile acidity by the method of Kosikowsky and Dahlberg (4), and soluble nitrogen by the method of Sharp as reported by Dahlberg and Kosikowsky (1). The samples of cheese were scored by the authors at the end of one month curing and bimonthly thereafter. The samples were analyzed bimonthly for volatile acids, soluble nitrogen and pH.

A considerable number of series of cheese were made with remarkably consistent results, and two series made on different days are presented to illustrate the results.

#### RESULTS

The manufacturing data (table 1) show that the rate of acid development with the *S. faecalis* starter was slower than with the commercial lactic starter, and the mixture of the two starters developed acid at a rate intermediate between those of the two cultures used singly. *S. faecalis* grows well in the salt concentration of Cheddar cheese, so the pH of all samples of cheese 1 day old was rather uniform at pH 4.9 to 5.1 (table 2), irrespective of considerable variations in acidity present in the whey when

the curd was milled. The composition of the cheese was uniform, but the salt contents were somewhat low (table 1). Most other batches of cheese in other experiments contained 1.5 to 2.0 per cent salt.

While the cheese curd was matting in the vats, it generally was possible to observe that curd containing *S. faecalis* matted slightly more rapidly and that the curd developed more of the stringy character of the meat of chicken breast. As the time approached for salting, the curd

TABLE 1  
The acidity development during the manufacture of the pasteurized-milk American Cheddar cheese and the percentage composition of the cheese made with lactic, lactic plus *S. faecalis*, and *S. faecalis* starters

Manufacturing data	Series 1—10464			Series 2—10468		
	Lactic	L. F. <sup>a</sup>	Faecalis	Lactic	L. F. <sup>a</sup>	Faecalis
Fresh milk, titr. acid. ....	0.15	0.15	0.15	0.16	0.16	0.16
pH .....	6.64	6.64	6.64	6.54	6.54	6.54
Amount of starter. (%) ....	2	1+1	2	2	1+1	2
Starter, titr. acid. (%) ....	0.74	.....	0.64	0.77	.....	0.61
Milk set, titr. acid. (%) ....	0.17	0.165	0.16	0.18	0.18	0.18
pH .....	6.41	6.40	6.42	6.40	6.38	6.42
Whey acid						
At cutting, titr. acid (%)	0.11	0.10	0.11	0.12	0.11	0.11
pH .....	6.35	6.38	6.42	6.33	6.40	6.46
Cooked, titr. acid. (%)	0.12	0.12	0.11	0.13	0.13	0.13
pH .....	6.22	6.28	6.35	6.21	6.32	6.32
Drawn, titr. acid. (%)	0.13	0.13	0.12	0.15	0.14	0.13
pH .....	6.11	6.17	6.29	6.05	6.05	6.18
Milling, titr. acid. (%)	0.52	0.45	0.38	0.50	0.38	0.25
pH .....	5.38	5.44	5.54	5.35	5.63	5.95
Cheese out of press						
Yield per cwt. milk (lb.)	10.4	10.4	10.4	11.0	11.5	11.9
Moisture (%) .....	35.9	36.9	36.3	34.9	35.8	36.9
Fat (%) .....	35.5	34.5	34.5	35.5	35.0	35.0
Salt (%) .....	1.18	1.25	1.42	1.39	1.27	1.45
Protein (%) .....	23.33	23.63	23.65	23.69	23.21	22.64

<sup>a</sup> L.F. = cheese containing 1% lactic starter and 1% *S. faecalis* starter.

containing *S. faecalis* developed a more pronounced odor of good Cheddar cheese curd. This odor of good cheese curd invariably was selected by several persons.

As the cheese cured there was a gradual increase in the pH to 5.13–5.29 for cheese held at 50° F. and to 5.19–5.33 for cheese held at 60° F. (table 2). The data are not extensive enough to show any conclusive difference in acidity due to storage temperature, but a higher pH at the

warmer curing temperature seems logical. Certainly, the acidity of the ripened cheese was not affected by the starters, for the range of pH on all samples of cheese when 6 months old was from 5.13 to 5.33.

The volatile acidity of the cheese increased more rapidly at 60° F. than at 50° F. (table 2), as would be expected. The commercial lactic starter produced more volatile acidity in the cheese than the *S. faecalis*

TABLE 2

*The pH, volatile acids, and water soluble proteins during curing of pasteurized-milk American Cheddar cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters*

Cheese no.		pH		Volatile acids		Water-soluble proteins	
		50° F.	60° F.	50° F.	60° F.	50° F.	60° F.
				(Ml. N acid/100 g.)		(%)	(%)
				1 day old			
Lactic	10464.....	4.99	.....	12.5	.....	1.45	.....
Lactic	10468.....	4.88	.....	12.5	.....	2.25	.....
L.F.	10464.....	5.05	.....	12.5	.....	1.59	.....
L.F.	10468.....	5.01	.....	16.7	.....	1.74	.....
Faecalis	10464.....	5.15	.....	12.1	.....	1.50	.....
Faecalis	10468.....	5.12	.....	17.5	.....	1.54	.....
				2 months old			
Lactic	10464.....	5.07	5.13	19.5	27.5	5.77	6.68
Lactic	10468.....	5.06	5.14	14.7	22.9	6.01	7.28
L.F.	10464.....	5.10	5.15	21.4	32.4	5.55	6.40
L.F.	10468.....	5.17	5.22	13.6	20.7	5.65	6.00
Faecalis	10464.....	5.15	5.12	16.6	18.6	5.03	6.24
Faecalis	10468.....	5.19	5.20	16.9	17.2	5.00	6.05
				4 months old			
Lactic	10464.....	5.13	5.22	28.4	35.5	7.78	8.32
Lactic	10468.....	5.10	5.24	18.1	35.0	7.07	8.26
L.F.	10464.....	5.03	5.24	27.1	35.6	7.55	8.10
L.F.	10468.....	5.15	5.29	18.6	31.7	6.46	7.84
Faecalis	10464.....	5.07	5.24	23.9	29.6	7.23	7.99
Faecalis	10468.....	.....	5.14	17.2	29.1	5.70	8.16
				6 months old			
Lactic	10464.....	5.19	5.19	27.1	43.3	7.85	8.33
Lactic	10468.....	5.20	5.29	27.2	39.0	8.00	9.26
L.F.	10464.....	5.29	5.28	29.4	39.2	7.49	8.95
L.F.	10468.....	5.22	5.33	22.5	36.5	7.02	8.67
Faecalis	10464.....	5.13	5.22	21.0	30.6	7.61	8.89
Faecalis	10468.....	5.19	5.31	15.8	26.2	7.10	8.61

starter, but the difference was not great. Cheese manufactured with *S. faecalis* starter and cured for 6 months at 50° F. showed practically no increase in volatile acidity. None of the samples of cheese was high in volatile acidity for, from a beginning of 12.1 to 17.5 ml. 0.1 N acid per 100 g., the volatile acidity value increased after 6 months at 50° F. up to 15.8 to 29.4 and at 60° F. up to 26.2 to 43.3.



As anticipated, the percentage of water-soluble protein increased more rapidly at 60 than at 50° F. (table 2). The two types of starters did not affect the increase in soluble proteins, which was reasonably uniform for all samples. The soluble protein in cheese cured 6 months at 50° F. ranged from 7.02 to 8.00, and that cured at 60° F. from 8.33 to 9.26.

The significant results from the use of *S. faecalis* starter are shown in the flavor scores and comments. Numerical scores were given to the nearest half point. All samples were graded as to intensity of Cheddar cheese flavor. Other comments on flavor were not made systematically, i.e., some excellent flavored samples of cheese were called excellent and others equally good were not so marked. All the comments made at scoring were entered in tables 3 and 4.

TABLE 3  
Flavor development in pasteurized-milk American Cheddar cheese made with lactic, lactic plus *S. faecalis*, and *S. faecalis* starters (Ripened at 50° F.)

Cheese no.	Total score	Flavor <sup>a</sup>		Body <sup>b</sup>	
		Score	Remarks	Score	Remarks
1 month old					
Lactic 10464	93.0	39.0	Mild —, flat	29.0	Corky, firm
Lactic 10468	92.5	39.0	Mild —, flat	28.5	Corky, sl. crumbly
L.F. 10464	95.0	40.5	Mild +, exc., raw	29.5	Waxy
L.F. 10468	94.0	40.0	Mild +, exc., raw	29.0	Waxy, sl. crumbly
Faecalis 10464	94.5	40.0	Mild +, exc.	29.5	Waxy
Faecalis 10468	95.0	40.5	Mild, exc., raw	29.5	Waxy
2.5 months old					
Lactic 10464	94.0	40.0	Mild —, clean	29.0	Waxy, sl. firm
Lactic 10468	93.0	39.5	Mild —, sl. curd	28.5	Sl. rubbery
L.F. 10464	95.5	41.0	Mild +, clean, raw	29.5	Waxy
L.F. 10468	95.0	41.0	Mild +, clean, exc.	29.0	Waxy
Faecalis 10464	95.5	41.0	Mild, clean	29.5	Waxy, sl. rubbery
Faecalis 10468	94.5	40.0	Mild, clean, exc.	29.0	Waxy, sl. rubbery
4.5 months old					
Lactic 10464	94.5	40.0	Medium —, exc.	29.5	Waxy, sl. firm
Lactic 10468	93.0	39.0	Mild, past.	29.0	Sl. waxy, sl. firm
L.F. 10464	94.5	40.0	Medium +, exc.	29.5	Waxy
L.F. 10468	94.5	40.0	Medium, raw	29.5	Waxy
Faecalis 10464	94.0	39.5	Medium, exc.	29.5	Waxy
Faecalis 10468	94.0	39.5	Medium —	29.5	Waxy
7 months old					
Lactic 10464	93.5	39.5	Medium —, sl. flat	29.0	.....
Lactic 10468	94.0	39.5	Medium	29.5	.....
L.F. 10464	95.0	40.5	Medium +	29.5	Waxy
L.F. 10468	95.0	40.5	Medium +	29.5	Waxy
Faecalis 10464	94.5	40.0	Medium	29.5	Waxy
Faecalis 10468	94.5	40.0	Medium	29.5	Waxy

<sup>a</sup> Flavor was scored with 45 as perfect. Intensity of flavor was rated mild—, mild, mild+, medium—, medium, medium+, sharp —, sharp.

<sup>b</sup> Body was scored with 30 as perfect.

Considering the cheese ripened at 50° F. (table 3), it will be noted that the cheese made with commercial lactic starter scored the lowest or possessed least flavor, whereas the cheese made with both lactic and *S. faecalis* starters scored the highest or possessed the most flavor of the

TABLE 4

*Flavor development in pasteurized-milk American Cheddar cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters*  
(Ripened at 60° F.)

Cheese no.		Total score	Flavor <sup>a</sup>		Body <sup>b</sup>	
			Score	Remarks	Score	Remarks
1 month old						
Lactic	10464	94.5	40.0	Mild	29.5	Sl. open
Lactic	10468	93.5	39.5	Mild	29.0	Firm
L.F.	10464	95.5	41.0	Medium, raw	29.5	Waxy
L.F.	10468	96.0	41.5	Medium, raw, exc.	29.5	Waxy
Faecalis	10464	94.5	40.0	Medium, raw	29.5	Waxy
Faecalis	10468	95.5	41.0	Medium, raw, exc.	29.5	Waxy
2.5 months old						
Lactic	10464	94.5	40.5	Mild +, past., flat	29.0	Sl. mealy
Lactic	10468	94.0	40.0	Mild, clean	29.0	Sl. crumbly
L.F.	10464	96.5	42.0	Medium +, clean,		
				exc., raw	29.5	Waxy
L.F.	10468	96.5	42.0	Medium, clean,		
				exc., raw	29.5	Waxy
Faecalis	10464	95.5	41.0	Medium, clean, raw	29.5	Waxy
Faecalis	10468	95.5	41.0	Medium —, exc.,		
				clean, raw	29.5	.....
4.5 months old						
Lactic	10464	94.5	40.0	Medium +	29.5	Waxy, sl. firm
Lactic	10468	91.5	38.0	Medium, burnt	28.5	Weak, sticky
L.F.	10464	95.0	40.5	Sharp —	29.5	Waxy
L.F.	10468	95.5	41.0	Medium +, exc.	29.5	Waxy
Faecalis	10464	95.0	40.5	Sharp —	29.5	Waxy
Faecalis	10468	94.5	40.0	Medium +, exc.	29.5	Waxy
7 months old						
Lactic	10464	93.0	39.0	Medium +, flat,		
				burnt	29.0	.....
Lactic	10468	91.5	38.0	Medium, burnt	28.5	Sl. crumbly
L.F.	10464	94.0	39.5	Sharp —	29.5	.....
L.F.	10468	93.5	39.5	Medium +	29.0	.....
Faecalis	10464	94.0	39.5	Sharp —	29.5	.....
Faecalis	10468	93.5	39.5	Medium +	29.0	.....

<sup>a</sup> Flavor was scored with 45 as perfect. Intensity of flavor was rated mild—, mild, mild+, medium—, medium, medium+, sharp—, sharp.

<sup>b</sup> Body was scored with 30 as perfect.

three lots. The difference in the scores was obvious for cheese of all ages up to 7 months, when scoring was discontinued. Furthermore, the intensity of the Cheddar flavor was greatest for cheese made with both lactic and *S. faecalis* starters and was least for cheese made with lactic

starter only. The flavor of the cheese with lactic starter usually was slightly flat, whereas with the use of both starters the flavor was full, clean, and often said to be excellent and like good raw milk cheese. The flavor of cheese made with *S. faecalis* starter alone closely resembled that of cheese made with combination starter, but neither quality nor intensity of flavor always was as good. It should be noted that when the flavor of the cheese with lactic starter was especially good, the quality closely approached that of cheese made with lactic and *S. faecalis* starters. All lots of cheese cured well without developing any off-flavors for 7 months at 50° F.

The waxy, mellow body of cheese made with the combination commercial lactic and *S. faecalis* starters, and with *S. faecalis* starters alone, was evident (table 3). The improved body of these samples of cheese could be detected even after 7 months of curing. The difference was much more noticeable than might be supposed by observing the numerical scores on body.

Cheese cured at 60° F. developed the same character as that cured at 50° F., except that changes occurred more rapidly. At 50° F. no cheese developed a sharp flavor in 7 months, but at 60° F., cheese made with *S. faecalis* starter alone or in combination with lactic starter was sharp in flavor in 4.5 months (table 4). The intensity of Cheddar flavor at 2.5 months at 60° F. approximated the intensity of flavor after 4.5 months at 50° F. This agrees closely with the work of Dahlberg and Marquardt (2). At 60° F. cheese made with lactic starter began to show some deterioration in flavor at 4.5 months and was obviously deteriorated at 7.5 months. The defect was a burnt or caramelized off-flavor. The cheese made with *S. faecalis* starter alone or in combination with lactic starter was excellent after 7 months at 60° F., but the flavor quality was slightly less than at 4.5 months of age. Observations of other batches of cheese show that about 4 months at 60° F. should be the maximum forced curing before cold storage at 40° F.

#### DISCUSSION

The scientific literature on Cheddar cheese of the last 50 years contains many articles showing that the use of a certain bacterial culture or enzyme or of a specific process of manufacture has intensified good flavor. With minor exceptions, none of these promising results ever has been successfully used commercially over an extended territory. Therefore, it is with some reluctance that the authors publish these data, but the results are of scientific value irrespective of commercial usage. This study is the first to embody successfully the use of large inoculations of a special culture into pasteurized milk to produce good, high flavored



American Cheddar cheese in a short curing period. Although the flavor is like that of raw milk cheese, it never develops the very "bitey" or "snappy" flavor that stings on the upper palate of the mouth, and is so typical of old raw milk cheese. Rather, the flavor is full and pronounced without being astringent or without having any rancid or other foreign flavor. Obviously, the milk must be of good flavor before pasteurization.

Good Cheddar cheese was made in these experiments with pasteurized milk containing only a commercial lactic starter. A better Cheddar cheese was made using *S. faecalis* starter alone, so this is positive proof that ordinary lactic starter is not necessary for making good Cheddar cheese. The best Cheddar cheese was made using both starters together, indicating symbiotic action among the bacteria in the two starters in producing a maximum of flavor.

In these experiments no time was given for the starter to develop before adding rennet, and the authors actually favor an hour at 86° F. for the starter to work in the milk before setting. Under such conditions about 0.5 to 0.75 per cent of lactic starter and 1 per cent of *S. faecalis* starter gave desired acid production.

*S. faecalis* has characteristics of special significance in cheese. It grows at 106° F. in the cheese vat and at 50° F. in the curing room. It ferments lactose rapidly enough to be used as a starter, providing a proper strain is selected and developed. It grows anaerobically in cheese, utilizing lactates as sources of energy. It is nonproteolytic and does not produce gas or objectionable flavors. It grows at the pH and the salt concentration present in cheese.

#### SUMMARY

A strain of *Streptococcus faecalis* which rapidly fermented lactose was isolated. It was used as a starter for American Cheddar cheese made from pasteurized milk of excellent quality.

The *S. faecalis* starter produced acid in milk somewhat slower than a commercial lactic starter but rapidly enough for cheese making. The cheese made with *S. faecalis* developed a normal acidity, slightly lower total volatile acidity, and the same water-soluble protein level as did cheese made with a commercial lactic starter. More Cheddar flavor of better quality developed in the cheese made with *S. faecalis*, and the body of the cheese was more mellow and waxy than the cheese made with lactic starter.

American Cheddar cheese with the best flavor of highest intensity was made by using both commercial starter and *S. faecalis* starter in the same pasteurized milk. The flavor was pronounced, clean, good Cheddar but not snappy.

*S. faecalis* starter hastened the ripening of Cheddar cheese. A well-ripened cheese of medium flavor intensity was produced in 4.5 months at 50° F. and in 2.5 months at 60° F. when *S. faecalis* starter was used with the usual lactic starter. With commercial lactic starter, the same approximate intensity of flavor, of lower quality, was developed in 7 months at 50° F. and in 4.5 months at 60° F. The results indicate that after these curing periods, the cheese should be held at cold curing temperatures.

## REFERENCES

- (1) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Flavor, Volatile Acidity, and Soluble Protein of Cheddar and Other Cheese. *J. Dairy Sci.*, 30: 165-174. 1947.
- (2) DAHLBERG, A. C., AND MARQUARDT, J. C. Ripening Cheese in Cans. *N. Y. State Agr. Expt. Sta. Tech. Bull.* 265. 1942.
- (3) DORN, F. L., AND DAHLBERG, A. C. Gas Production by Cheddar and Limburger Cheeses Ripened in Tin Cans. *N. Y. State Agr. Expt. Sta. Tech. Bull.* 266. 1942.
- (4) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. *J. Dairy Sci.*, 29: 861-871. 1946.
- (5) SHERMAN, J. M. The Streptococci. *Bact. Revs.* 1: 3-97. 1937.
- (6) WHITE, J. C., AND SHERMAN, J. M. Occurrence of Enterococci in Milk. *J. Bact.*, 48: 262. 1944.
- (7) WILSON, H. L. Making American Cheddar Cheese of Uniformly Good Quality. *U. S. Dept. Agr. B.D.I.M.* 947. Nov., 1942.
- (8) WILSON, H. L., HALL, S. A., AND JOHNSON, W. T., JR. Relationship of Curing Temperature to Quality of American Cheddar Cheese. *J. Dairy Sci.*, 24: 169-178. 1941.

# THE GROWTH AND SURVIVAL OF *STREPTOCOCCUS FAECALIS* IN PASTEURIZED MILK AMERICAN CHEDDAR CHEESE<sup>1</sup>

F. V. KOSIKOWSKY AND A. C. DAHLBERG

Department of Dairy Industry, Cornell University, Ithaca, New York

In a previous paper (1) it was shown that the addition of *Streptococcus faecalis* starter to pasteurized cheese milk increased the rate of ripening and improved the flavor of American Cheddar cheese. Such an effect naturally directed the attention of the authors to the growth characteristics of this organism in cheese.

It is well known that the bacteria normally found in commercial lactic cheese starters do not survive for any great length of time in cheese. *S. faecalis*, on the other hand, is considered to be a rugged type of organism, able to survive and grow under conditions which would soon destroy many other types. As American Cheddar cheese in many respects affords conditions unfavorable for growth of most bacteria, it would be interesting to observe the degree of adaptation that *S. faecalis* could make in such an environment. That this organism commonly is found in cheese has been noted by several investigators. Sherman and Stark (5) found *S. faecalis* in 1-day-old Swiss cheese, while Foster *et al.* (2) found large numbers of these bacteria in ripening Brick cheese. Tittler *et al.* (6) stated that enterococci were one of the predominant bacterial types in ripening Cheddar cheese made from pasteurized milk.

Up to the present, very little study has been made of the course of growth and of the survival period of *S. faecalis* in Cheddar cheese. Data of this nature should aid in an understanding of the effect of this organism upon cheese flavor development as well as provide general information which will be required as knowledge of the relationship of *S. faecalis* to foods becomes more apparent. A study covering the foregoing phases was conducted.

## EXPERIMENTAL METHODS

Methods used consisted of the total plate count using standard tryptone-glucose-extract-skim milk agar, a selective medium plate count for enterococci, and conventional physiological identification tests for enterococci. The selective medium was that developed by White and Sherman (7) for the determination of enterococci in milk. It contains 0.5 per

Received for publication December 10, 1947.

<sup>1</sup> This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Lois Phelps and Miss Catherine Verwoert for making many of the chemical analyses.



cent glucose, 0.5 per cent tryptone, 0.5 per cent yeast extract, 1.5 per cent agar, 0.03 per cent sodium azide, and 325 Oxford units of penicillin per l. Tests employed for the identification of the enterococcus group were those suggested by Sherman (4) as being very important for differential purposes. They included growth at 10° C. and 45° C., rapid reduction in litmus milk, and growth in broth containing 6.5 per cent sodium chloride. In addition, microscopic observations were made. All plates were incubated at 32° C. for 4 days. Samples for plating were prepared by aseptically grinding 3 g. of cheese with 27 ml. of warm 2 per cent sodium citrate solution until the cheese was well emulsified. Dilutions then were made from this cheese solution.

#### RESULTS

*Total and selective medium counts of milk, starters, and fresh curd.* Results shown in this work were obtained on a series of three cheese. All cheese were made from milk pasteurized at 143-145° F. for 30 minutes, with the raw milk being obtained from the Cornell University herd. Twelve hundred pounds of milk were divided into three equal portions and made into cheese. The cheese obtained was the series L 10468, LF 10468 and F 10468 referred to in a previous paper (1) in which L was made with 2 per cent commercial lactic starter, LF with 1 per cent commercial lactic starter and 1 per cent *S. faecalis* starter, and F with 2 per cent *S. faecalis* starter. The cheese were ripened at 50 and at 60° F.

Bacterial counts, using standard agar and penicillin-sodium azide medium, were made of the original raw and pasteurized milk, and starters. Although the selective medium was used to separate the enterococci, no attempt was made at this stage to identify the enterococcus colonies by additional tests. Results of these counts are shown in table 1. The two different types of starters showed relatively high total bacterial counts. The regular commercial starter showed a total bacterial count of 350 million per ml., while the *S. faecalis* starter had a total bacterial count of 950 million per ml. Results with the selective medium showed that no enterococci were present in the regular commercial starter, while approximately 94 per cent of the total count of the *S. faecalis* starter grew in the selective medium.

The original raw milk was of high quality, having a total bacterial count of 5,700, while the pasteurized milk had a low bacterial count of 500 per ml. The numbers of bacteria growing on the selective medium were very small in either milk. The total bacterial counts after the addition of the starters ranged from 5 million to 16 million, whereas the selective medium counts ranged from 70 to 12 million.

The next counts were taken on the cheese curds just before salting.

Results are listed in table 2 under 0 days. Beginning with this section and continuing through, with a few exceptions, 20 colonies from the plates of each cheese were isolated and cultured from plates containing the selective medium. These bacteria then were identified as to whether or not they were enterococci, using the tests enumerated previously.

*Total and selective medium counts of cheese ripened at 50° F.* Of the cheese ripened at 50° F. (table 2), cheese *L*, containing 2 per cent lactic starter, had its highest total bacterial count, 300 million per g., within the first 2 days, after which the bacterial population decreased rapidly to the

TABLE 1

*Bacterial counts on starters and cheese milk containing commercial lactic starter organisms and S. faecalis organisms*

Milk	Total bacterial counts per ml. on standard agar	Bacterial counts per ml. on penicillin-azide agar
Commercial starter .....	350,000,000	1
<i>S. faecalis</i> starter .....	950,000,000	890,000,000
Raw milk .....	5,700	320
Past. milk (143-145° F. for 30 min.) .....	500	50
<i>L</i> 10468—past. milk set at 86° F. containing 2% com. starter .....	5,000,000	70
<i>LF</i> 10468—past. milk set at 86° F. containing 1% com. starter and 1% <i>S. faecalis</i> starter .....	11,500,000	6,400,000
<i>F</i> 10468—past. milk set at 86° F. containing 2% <i>S. faecalis</i> starter .....	16,000,000	12,000,000

low total count of 1,200,000 per g. at the end of 23 days and then gradually increased to a total count of 26 million at the end of 180 days. Cheese *LF*, containing 1 per cent lactic starter and 1 per cent *S. faecalis* starter, had its highest total count, 1,150 million per g., at the time of salting the curd. The number of bacteria then decreased very slowly over the ripening period of 180 days to a low of 305 million. Cheese *F*, containing 2 per cent *S. faecalis* starter, on the other hand, had a high total count of 1,790 million at the time of salting, but this high count was maintained at the same level for 60 days, after which it slowly decreased to 855 million at the end of 180 days.

When the selective penicillin-azide medium for enterococci was used on the cheese ripened at 50° F., the following results, outlined in table 2, were obtained. The selective medium bacterial count for cheese *L* was lowest during the first 2 days. Just prior to salting of the cheese, the bacterial

count was 300, with 18 of the 20 colonies being identified as enterococci. From this low point, the bacteria in this control cheese increased to a peak of 23 million per g. However, it can be seen clearly from the summary of identification tests (table 2) that the increase in numbers was not a

TABLE 2

*Bacterial counts of pasteurized-milk American Cheddar cheese (Series 10468) made from commercial starter, S. faecalis starter, and a mixture of the two, and ripened at 50° F.*

Cheese <sup>a</sup>	Age	Total bacterial count per g. on standard agar	Bacterial count per g. on penicillin-azide agar	Positive identification of enterococci
	(days)			(from 20 picked colonies)
L	0	300,000,000	300	18
LF	0	1,150,000,000	520,000,000	19
F	0	1,790,000,000	1,370,000,000	20
L	2	320,000,000	100	.....
LF	2	1,070,000,000	490,000,000	.....
F	2	1,890,000,000	1,100,000,000	.....
L	11	60,000,000	5,000	4
LF	11	920,000,000	500,000,000	19
F	11	1,750,000,000	1,310,000,000	20
L	23	1,200,000	14,000	.....
LF	23	686,000,000	389,000,000	.....
F	23	1,560,000,000	1,076,000,000	.....
L	34	2,900,000	295,000	2
LF	34	740,000,000	390,000,000	19
F	34	1,810,000,000	1,065,000,000	20
L	60	11,000,000	2,700,000	6
LF	60	480,000,000	345,000,000	20
F	60	1,630,000,000	1,085,000,000	19
L	120	44,000,000	23,000,000	0
LF	120	366,000,000	290,000,000	19
F	120	970,000,000	740,000,000	18
L	180	26,000,000	11,000,000	0
LF	180	305,000,000	250,000,000	19
F	180	855,000,000	675,000,000	20

<sup>a</sup> L = 2% regular lactic starter in cheese milk.

LF = 1% regular lactic starter in 1% *S. faecalis* starter in cheese milk.

F = 2% *S. faecalis* starter in cheese milk.

result of an increase in enterococci but rather of another type or types of bacteria able to multiply on the selective medium. Further examination of these organisms showed them to be of the genus *Lactobacillus*.

In cheese LF, the highest number of enterococci, 520 million per g., was found in the curd prior to salting. The numbers of these bacteria



were maintained at almost 70 per cent of this level for 60 days, while at the end of 180 days there was a decline of about 50 per cent. Almost all the bacteria isolated were of the enterococcus group.

Cheese *F* at 50° F. had the highest enterococcus count, 1,370 million per g., just before the curd was salted, and this count was maintained at

TABLE 3

*Bacterial counts of pasteurized-milk American Cheddar cheese (Series 10468) made from commercial starter, S. faecalis starter and a mixture of the two, and ripened at 60° F.*

Cheese <sup>a</sup>	Age	Total bacterial count per g. on standard agar	Bacterial count per g. on penicillin-azide agar	Positive identification of enterococci
	(days)			(from 20 picked colonies)
L	0	300,000,000	300	18
LF	0	1,150,000,000	520,000,000	19
F	0	1,790,000,000	1,370,000,000	20
L	2	.....	.....	.....
LF	2	.....	.....	.....
F	2	.....	.....	.....
L	11	50,000,000	36,000	3
LF	11	960,000,000	450,000,000	19
F	11	1,680,000,000	1,330,000,000	20
L	23	3,200,000	2,700,000	.....
LF	23	680,000,000	420,000,000	.....
F	23	1,490,000,000	1,030,000,000	.....
L	34	14,000,000	5,500,000	1
LF	34	630,000,000	385,000,000	18
F	34	1,600,000,000	1,020,000,000	20
L	60	50,000,000	26,000,000	0
LF	60	450,000,000	305,000,000	18
F	60	875,000,000	750,000,000	20
L	120	61,000,000	28,000,000	0
LF	120	150,000,000	115,000,000	18
F	120	530,000,000	450,000,000	19
L	180	35,000,000	13,500,000	0
LF	180	61,000,000	36,000,000	13
F	180	165,000,000	90,000,000	20

<sup>a</sup> L = 2% regular lactic starter in cheese milk.

LF = 1% regular lactic starter in 1% *S. faecalis* starter in cheese milk.

F = 2% *S. faecalis* starter in cheese milk.

the same level for 60 days, after which it decreased to 675 million at the end of 180 days. Practically all the colonies isolated by means of the selective medium belonged to the enterococcus group, and, as only *S. faecalis* was added, presumably all or almost all of this species made up the bacterial count of cheese *F*.

*Total and selective medium counts of cheeses ripened at 60° F.* The bacterial counts of cheese ripened at 60° F. compared to those of the same lots of cheese ripened at 50° F. showed many similar trends (table 3). In control cheese *L*, the total bacterial count showed a rapid decrease from a high of 300 million to a low of 3 million per g. at the end of 23 days. This was followed by a steady increase until at the end of 120 days the total bacterial count was up to 61 million. Two months later the numbers of bacteria had decreased to 35 million. In cheese *LF* a high total bacterial count of 1,150 million was obtained on the curds just prior to salting. The total count then gradually decreased to 450 million at the end of 60 days, while at the end of 180 days the bacterial count had gone down to 61 million. Cheese *F* showed results in line with those exhibited by cheese *LF*, going from a high of 1,790 million in the curd to a low of 165 million at 180 days.

Selective medium counts made on these cheese ripened at 60° F. (table 3), showed control cheese *L* with a low initial count of 300, followed by a steady increase to 28 million at the end of 120 days and a drop to 13,500,000 at 180 days. This increase in cheese *L* was not due as much to enterococci as to lactobacilli. On the other hand, cheese *LF* had its highest enterococcus count just before salting, 520 million per g., and this population decreased to 36 million at 180 days. At the end of 60 days of ripening, 305 million enterococci per g. still were present. In this connection, cheese *F*, with *S. faecalis* starter only, showed counts which were maintained for long periods of time. Starting with an initial selective medium count of 1,370 million, this cheese still had a count of 750 million at the end of 60 days, and then dropped to 90 million at 180 days.

A comparison of tables 2 and 3 shows that at 50 and at 60° F. the trends of bacterial growth and survival were very similar; the difference that existed showed up as a more rapid decline in bacterial population at 60° F., a result which was expected.

A duplicate experiment made on cheese manufactured a month later produced data of strikingly similar nature. These data are not included in this paper because they would only provide repetition of the initial observations.

#### DISCUSSION

A study was made of the growth and survival of *Streptococcus faecalis* in pasteurized milk American Cheddar cheese over a 6-month ripening period at 50 and at 60° F. Control cured cheese made from pasteurized milks and containing only regular lactic cheese starter had the lactobacilli as their predominating organisms. These results do not agree with those of Tittsler *et al.* (6), who stated that enterococci were the predom-

inating organisms in pasteurized milk cheese. However, it is pointed out that the milk used in the present study was of very high quality, and cheese made commercially from pasteurized milk was not included. Development of lactobacilli in large numbers late in the ripening period of Cheddar cheese was noted very early by Hastings *et al.* (3) and by other investigators.

The observation that the selective medium developed by White and Sherman (7) for the separation of enterococci actually allowed bacteria of the lactobacillus group to grow confirms the earlier findings of White and Sherman (8). These investigators found that in cheese certain species of lactobacilli were able to grow in the selective medium. Where enterococci are predominant, this method of selection is very useful and surprisingly consistent in its ability to recover the organism. In the work involving cheese *F*, where there was a vast number of *S. faecalis* organisms, the selective medium was able to recover on the average about 73 per cent of the total bacterial count as enterococci. This is a good recovery when one considers the many opportunities that exist for the elimination of the less resistant bacteria in work of this nature.

*Streptococcus faecalis* proved able to adapt itself well to the environment provided by Cheddar cheese when added as a starter. Its best growth occurred in the milk and curd up to salting, and it was able to grow and survive in large numbers after 180 days of ripening at 50 and at 60° F. This characteristic resistance to destruction in cheese further strengthens the authors' belief that this organism is instrumental in developing increased flavor in cheese.

#### SUMMARY

Three lots of milk pasteurized at 143–145° F. for 30 minutes were made into American Cheddar cheese. These lots contained, respectively, 2 per cent commercial lactic starter, 1 per cent commercial lactic starter plus 1 per cent *Streptococcus faecalis* starter, and 2 per cent *S. faecalis* starter. A selective penicillin-azide medium was used to count and isolate the enterococci.

In 1-day-old cheese made with commercial lactic starter, the number of bacteria growing on the selective medium was small, 300 per ml., but these gradually increased to 23 million per ml. at the end of 120 days at 50° F., and to 28 million per ml. after 120 days at 60° F. At the end of 180 days the counts on the selective medium had decreased to 11 million and 13 million per ml. at 50 and 60° F., respectively. In this cheese most of the increase was due to lactobacilli and not to enterococci.

When *S. faecalis* was used as a starter for pasteurized milk American Cheddar cheese, the highest enterococcus count was found to exist in the



cheese curds just prior to salting, the count being 500 million per g. for cheese containing 1 per cent commercial lactic starter plus 1 per cent *S. faecalis* starter, and 1,370 million per g. for the cheese containing 2 per cent *S. faecalis* starter.

*S. faecalis* was able to grow and survive in Cheddar cheese in large numbers for a considerable period of time, both at 50 and 60° F. At 50° F., cheese made with both lactic and *S. faecalis* starters still gave counts of 345 million per g., and cheese made with *S. faecalis* starter gave counts of 1,085 million per g. at the end of 60 days, whereas at 60° F. the former cheese contained 305 million per g. and the latter cheese contained 750 million per g. at the end of 60 days. At the end of 180 days the numbers of bacteria in the cheese growing on the selective medium had decreased, although considerable numbers still were present.

#### REFERENCES

- (1) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Development of Flavor in American Cheddar Cheese Made from Pasteurized Milk with *Streptococcus faecalis* Starter. *J. Dairy Sci.*, 31: 275-284. 1948.
- (2) FOSTER, E., GAREY, J. C., AND FRAZIER, W. C. The Bacteriology of Brick Cheese. III. The Bacteria Involved in Ripening. *J. Dairy Sci.*, 25: 323-333. 1942.
- (3) HASTINGS, E. G., EVANS, A. C., AND HART, E. B. Studies on the Factors Concerned in the Ripening of Cheddar Cheese. *Wis. Agr. Expt. Sta. Res. Bull.* 25. 1912.
- (4) SHERMAN, J. M. The Streptococci. *Bact. Revs.* 1: 3-97. 1937.
- (5) SHERMAN, J. M., AND STARK, PAULINE. Streptococci Which Grow at High Temperatures. *J. Bact.*, 22: 275-285. 1931.
- (6) TITSLER, R. P., GEIB, DONNA S., SANDERS, G. P., WALTER, H. E., SAGER, O. S., AND LOCHRY, H. R. The Effects of Quality and Pasteurization of Milk on the Bacterial Flora and Quality of Cheddar Cheese. *J. Bact.*, 51: 590. 1946.
- (7) WHITE, J. C., AND SHERMAN, J. M. Occurrence of Enterococci in Milk. *J. Bact.*, 48: 262. 1944.
- (8) WHITE, J. C., AND SHERMAN, J. M. Personal communication. 1946.

## THE TYRAMINE CONTENT OF CHEESE<sup>1</sup>

F. V. KOSIKOWSKY AND A. C. DAHLBERG

*Department of Dairy Industry, Cornell University, Ithaca, New York*

The process of cheese ripening produces a variety of nitrogenous decomposition products, some of which must exert an important influence upon the ripening process. Yet relatively little is known concerning the nature of many of these compounds, their concentration in cheese, their specific rôle in ripening, and their nutritional value. For example, information is relatively meager on the amino acids freed in cheese during ripening, and even less is known concerning the respective amines which may be formed from these amino acids.

This paper deals with a small portion of this complex problem in that a quantitative study on cheese was made of the amine derived from tyrosine. This breakdown product from tyrosine is *p*-hydroxyphenylethylamine and very commonly is referred to as tyramine. No information has been available to indicate its quantitative concentration in cheese, with the single exception of an Emmenthal cheese.

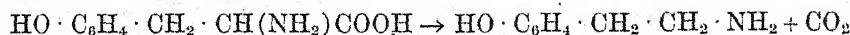
Tyramine is an alkaloid of the aromatic amine type. It can be produced by heating tyrosine with a high boiling solvent such as diphenylamine or by bacterial decomposition of tyrosine. Often it occurs in decaying protein and it also is found in ergot and mistletoe. Tyramine has a boiling point of 179–181° C. (8 mm.) and a melting point of 161° C. When injected subcutaneously or intravenously, it has the property of contracting the peripheral blood vessels, thus causing an increase in blood pressure, and for this reason it is used rather frequently in medicine. Gale (6) found the optimum production of tyramine by bacterial cells to occur at pH 4.5 to 5.5, which is in the pH range of normal American Cheddar cheese.

Tyramine was first discovered in cheese in 1903 by Van Slyke and Hart (8) in their research to show the source of carbon dioxide in cheese. They made two small batches of Cheddar cheese from fresh milk and from fresh milk to which chloroform had been added. Of specific interest in their study was the accumulation of relatively large amounts of tyrosine and no tyramine in the chloroformed cheese (a low acid cheese) after curing for 32 weeks at 15.5° C. (60° F.) as compared with lesser amounts of tyrosine and positive tests for tyramine in the normal cheese. This conversion of tyrosine to tyramine was thought to be due to bacteria. They cited the research of Emerson (5), published the previous year, which established

Received for publication December 10, 1947.

<sup>1</sup> This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mr. Allan Leventhal for his aid in some of the chemical analyses.

that the aqueous extract of the pancreas converted tyrosine into tyramine with liberation of carbon dioxide by the following reaction:



An abnormal Emmenthal cheese was found in 1909 by Winterstein and Küng (10) to contain tyramine. The authors did not state the reason for considering this cheese to be abnormal, but they found the tyrosine content to be abnormally low for a well-ripened cheese. They believed that bacteria probably converted the tyrosine into tyramine. Later Winterstein (9) found traces of tyramine in a skim milk Emmenthal cheese.

In 1914 Ehrlich and Lange (4) reported the presence of tyramine in samples of Roquefort, Camembert and Emmenthal cheese. The tyramine content of the Emmenthal cheese was found to be 1.08 g. in 1.8 kg. of cheese, or 0.06 per cent. When the cheese was inoculated into a bacterial culture medium containing tyrosine, tyramine was produced. A culture was isolated which produced tyramine by this method, but it did not produce tyrosol or *p*-hydroxyphenyllactic acid. This culture which produced acid in milk belonged to the colon group of bacteria. The literature includes the analysis for tyramine of only one Cheddar, one Roquefort, one Camembert and three Emmenthal cheeses. All samples tested showed the presence of tyramine, but of these six samples, only one, an Emmenthal cheese, was analyzed quantitatively.

Methods for the separation and estimation of tyramine have been known for some time, but only in recent years have newer methods been introduced which can be applied on a large scale to the estimation of this compound in such products as cheese. Henze (7) developed a quantitative method for obtaining and separating tyrosine and tyramine from cephalopods by ether extraction. With this method he isolated tyrosine and tyramine from the salivary gland of *Octopus macropus* and determined the compounds colorimetrically by the Millon reaction. Recently Bellamy and Gunsalus (1, 2), by using a continuous ether extractor and a colorimeter, adapted and applied this method to the determination of tyrosine and tyramine in bacterial cultures in their study on tyrosine decarboxylase systems. Utilizing the knowledge obtained by the foregoing investigators, an applied method for determining tyramine in cheese was evolved. With this method it was possible to test quantitatively a large number of cheeses.

#### EXPERIMENTAL METHODS

The principle of this method is that the phenolic hydroxyl group, which is characteristic for tyrosine and tyramine, will react to form a positive Millon test under the proper conditions. Separation of tyramine from tyrosine is based on the fact that tyramine can be extracted by ether under mildly alkaline conditions, while tyrosine is insoluble in ether. Both are



insoluble in acid ether, while phenols are soluble. The method as applied to cheese is described.

*Preparation of sample.* Fifteen grams of cheese were ground in a mortar with a small amount of warm ( $45^{\circ}\text{C}.$ ) 2 per cent sodium citrate solution. After the cheese was well emulsified, the solution was transferred quantitatively to a 250-ml. volumetric flask and enough sodium citrate solution was added to bring it to the 250-ml. mark after cooling to  $25^{\circ}\text{C}.$  The contents of the volumetric flask were transferred to a standard 300-ml.

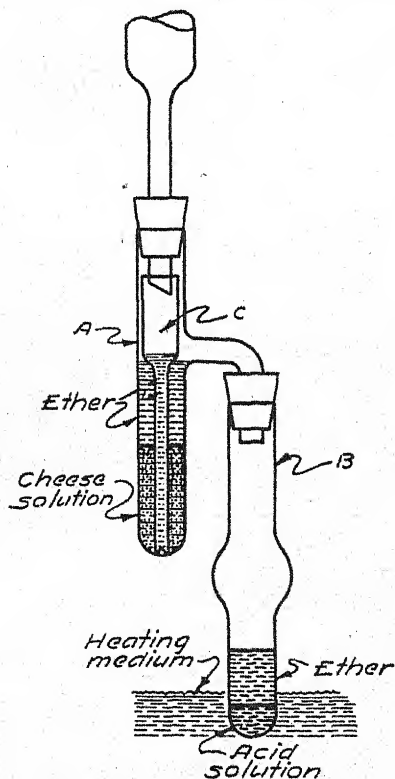


FIG. 1. A continuous extractor for separating tyramine from tyrosine. A, extraction tube ( $200 \times 25$  mm.); B, receiver tube ( $200 \times 25$  mm.); C, glass thimble. Condenser capacity—300 mm.

pyrex flask and heated to  $80^{\circ}\text{C}.$  for 15 minutes in a water bath to destroy the decarboxylases and then cooled to  $25^{\circ}\text{C}.$  After cooling, an 18-g. sample obtained by using a calibrated pipette was transferred to the extraction tube (A) of the continuous ether extractor shown in figure 1. This extractor, very similar to the one proposed by Wooley (11), was used by Bellamy and Gunsalus (1, 2) in their work on bacterial decarboxylases.

*Acid extraction of cheese.* To the receiver tube (B, fig. 1) of the ex-

tractor, 5 ml. of M/50 sulfuric acid were added. This acid solution is used to trap the tyramine when it comes over with the ether. The glass thimble (C, fig. 1) was placed in the extraction tube and then ethyl ether carefully was added to both the extractor and receiver tube. This was done until a 3-cm. layer accumulated in the receiver and until the ether just reached the side arm in the extractor tube. The two sections were attached to each other by means of cork connections and then attached to a condenser. Gentle heating of the ether in the receiver tube was accomplished by the use of an oil bath. Extraction under acid conditions was carried on for 5 hours to remove a large portion of the fat and fatty acids and any phenols that might be present. This extraction, due to the influence of the sodium citrate, was carried at slightly above the pH of most normal Cheddar cheese, or about pH 5.5-5.8.

*Alkali extraction of cheese.* The receiver tube was emptied, washed, and again filled to its former level with 5 ml. of M/50 sulfuric acid and sufficient ethyl ether and attached to the extraction section. The entire extraction unit then was taken from the oil bath, and enough of a solution of 10 per cent sodium carbonate was added to the thimble in the extractor tube to make the solution to be extracted slightly alkaline to phenolphthalein. The quantity required varied from 0.4 to 1.0 ml. No phenolphthalein actually was added to the extractor tube as it would be extracted by ether and would give a positive test. To find the proper amount of alkali required without adding phenolphthalein to the unit, an Erlenmeyer flask containing 18 g. of the cheese solution and phenolphthalein was used and the solution in it neutralized. The same amount of alkali required for this preliminary neutralization was added to the thimble. Extraction was resumed and continued until all the tyramine was obtained. This usually took about 42 hours, depending on the nature of the cheese. To assure complete extraction, analyses were made on the contents of the receiver tube after 30 hours and then at 12-hour intervals until the pink color no longer was produced.

After each of these extraction periods the receiver tube was disengaged from the extractor tube and the entire ether-acid mixture was cooled slightly and poured slowly into a graduated test tube. The test tube was placed into an oil bath and the ether carefully boiled off. The acid solution remaining was cooled to 25° C., and the volume noted and tyramine analyses made. The receiver tube again was filled with acid solution and ether, and the extraction resumed.

*Measurement of color.* One milliliter of the acid solution containing the tyramine was pipetted into a colorimeter tube (calibrated 18×150 mm. pyrex test tube). Three milliliters of 95 per cent acetic acid were added, followed by 2 ml. of a mercuric sulfate reagent (10 per cent mercuric sulfate in 5 per cent sulfuric acid) and the tube well agitated. This mixture

was heated for 3 minutes in boiling water, cooled and mixed. The tube then was placed in a Coleman no. 11 spectrophotometer and read at 500  $\gamma$  against a reagent blank set at  $G = 100$ . After this turbidity reading ( $L_1$ ) if any, was recorded, 1.0 ml. of fresh 0.5 per cent sodium nitrate (prepared fresh daily) was added and well mixed, and the tube read at 500  $\gamma$  after 15 minutes at room temperature against a reagent blank to which sodium nitrate solution had been added. The second reading with the color-producing compound was labeled  $L_2$ .

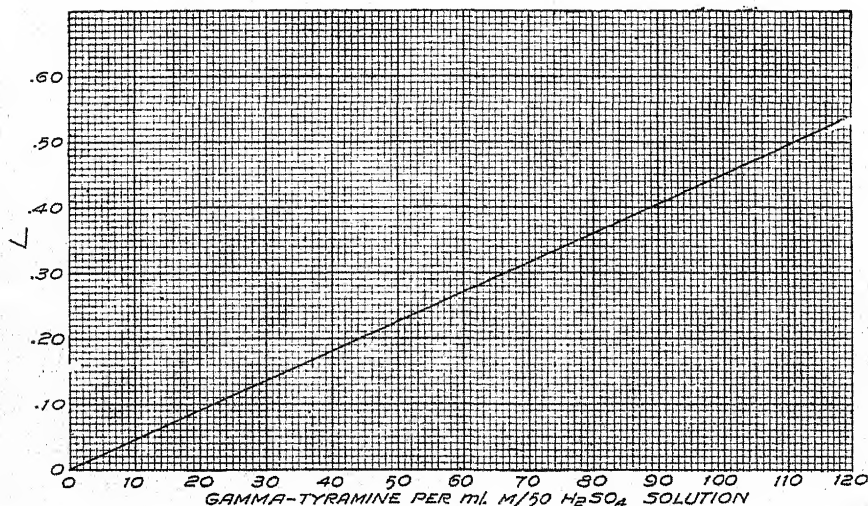


FIG. 2. Standard curve for tyramine using the Coleman spectrophotometer.

*Calculations.* Calculations required for this method are as follows:

$$L = 2 - \log G$$

$$L_2 - L_1 = L \text{ (proportional to tyramine concentration)}$$

$G$  = galvanometer reading

$L_1$  = value of turbidity

$L_2$  = value for color

To find the amount of tyramine in the sample, one of two things can be done. Reference can be made to the standard curve (fig. 2), or the tyramine content can be calculated by using a constant  $K$ , which has been obtained at different levels. To use  $K$ , the standard curve must go through the origin.

$$K = \frac{\text{gamma in standard sample}}{L \text{ for same sample}}$$

$$\text{Gamma in standard sample} = KL$$

The final standard is brought to gammas of tyramine per gram of cheese, after considering all dilution factors. In this work all values are based on tyramine and not tyramine hydrochloride.



## RESULTS

*The recovery of added tyramine from cheese.* In order to test the ability of this applied method to recover tyramine from cheese effectively and at the same time to prevent tyrosine from being extracted, a number of recovery experiments were performed. The results from several experimental trials indicated that when pure tyrosine was added to water or to a cheese solution, no amount of extraction, either at an acid or alkaline pH, would bring over the tyrosine. On the other hand, when various amounts of tyramine in the form of tyramine hydrochloride were added to a cheese solution, which then was made mildly alkaline with 10 per cent sodium carbonate, it was found (table 1) that practically all the added tyramine was recovered under the conditions of extraction and color

TABLE 1

*The recovery of added tyramine from cheese (5468 Fe)*  
(5-hr. acid ether extraction + 40-hr. alkali ether extraction)

Tyramine added	Tyramine found in cheese	Tyramine recovered
( $\gamma/g.$ )	( $\gamma/g.$ )	(%)
0	1427	.....
790	2191	97.0
1580	3008	100.0

estimation previously described. In this recovery experiment 5 hours of acid ether extraction and 40 hours of alkali ether extraction were required for complete recovery. For some cheese solutions where smaller amounts of tyramine were added, complete recovery was attained in a shorter length of time.

*The tyramine content of 25 Cheddar cheeses.* Analyses for tyramine were conducted on 25 commercial American Cheddar cheeses. These samples were obtained from New York, Wisconsin, Illinois, and Missouri and consisted of both raw and pasteurized milk cheese. The ages of these cheese ranged from 2 months to 3 years. No attempt was made to show here the effect of such factors as treatment of milk or cheese and the effect of age upon the tyramine content, as these topics will form the basis of subsequent papers. The high, average, and low tyramine concentrations of 25 American Cheddar cheeses are shown in table 2.

The tyramine content of Cheddar cheese can be much greater than that listed in the table. One very old Cheddar cheese, not included in the group of 25 cheeses because it was not manufactured in a commercial plant, was found to contain 2,330  $\gamma$  of tyramine per g., or 0.223 per cent. Data shown

here make it quite apparent that practically all commercial American Cheddar cheese must contain tyramine in a wide range of concentration.

*The tyramine content of miscellaneous types of cheese.* A number of cheeses representing diversified types were subjected to analyses for tyramine. The results of these analyses are shown in table 3. All samples within a type variety were purchased from different sources and at different times of the year. It was assumed from the beginning that the values shown here do not necessarily typify any particular variety of cheese, as factors tending to shift these values always are present. Data shown in table 3, however, do give some idea as to the tyramine content of an assorted group of cheeses. Here again, as with the Cheddar cheeses, a wide range exists not only between varieties but also between samples of similar types. The smallest amount of tyramine, 48  $\gamma$  per g., occurred in a Roquefort, while the largest amount, 1,683  $\gamma$  per g., occurred in a Liederkrantz cheese.

TABLE 2

*The concentration of tyramine in twenty-five commercial Cheddar cheeses*

Cheese	Tyramine	Tyramine
	( $\gamma$ /g.)	(%)
Highest .....	1199	0.1199
Lowest .....	25	0.0025
Av. ....	384	0.0384

Two different samples of Liederkrantz both gave high concentrations of tyramine. This is undoubtedly a result of the character of this cheese, where early and extensive decomposition of proteins occurs. The Limburger sample, which is considered similar to Liederkrantz in its decomposition properties, had a relatively low value. Further examination of this sample showed it to be very atypical in that it was not broken down in body and that it resembled a Brick cheese more than a Limburger.

*The isolation, purification and identification of the dibenzoyl derivative of tyramine from Cheddar cheese.* A series of tyramine extractions was conducted on several Cheddar cheeses which were considered to have large concentrations of tyramine. Sufficient extractions were made from fresh samples of cheese to provide a volume of 500 ml. of N/50 sulfuric acid calculated to contain about 35 mg. tyramine. The acid solution (about pH 1) then was evaporated *in vacuo* to approximately 40 ml., washed with ethyl ether to remove any fat, and then centrifuged to remove other impurities.

The method for obtaining a dibenzoyl derivative of tyramine as described by Gale (6) was followed. Solid sodium bicarbonate was added until the pH of the solution was brought to 7.5-8.0 and the mixture was

cooled in ice to 10–12° C. Benzoyl chloride then was added, with vigorous shaking, a few drops at a time until about 3 mol. equivalents had been added, the pH being maintained in the region of 8 by the addition of solid sodium bicarbonate. This mixture was left overnight in a cold room. The following day a precipitate had formed and was removed by centrifuging. It was purified by extracting with hot absolute alcohol. The dibenzoyl

TABLE 3  
*The concentration of tyramine in an assorted  
group of cheeses*

Cheese	Tyramine	Tyramine
	( $\gamma/g.$ )	(%)
Edam <sup>a</sup> .....	214	0.0214
Edam .....	100	0.0100
Roquefort <sup>a</sup> .....	48	0.0048
Blue .....	49	0.0049
Blue .....	266	0.0266
Limburger .....	204	0.0204
Liederkrantz .....	1226	0.1226
Liederkrantz .....	1683	0.1683
d'Oka <sup>a</sup> .....	310	0.0310
d'Oka <sup>a</sup> .....	158	0.0158
Gouda <sup>a</sup> .....	95	0.0095
Gouda .....	54	0.0054
Brick .....	194	0.0194
Munster .....	110	0.0110
Swiss .....	50	0.0050
Swiss .....	434	0.0434
Romano <sup>a</sup> .....	197	0.0197
Argenti <sup>a</sup> .....	188	0.0188
Camembert .....	125	0.0125
Mild process <sup>b</sup> .....	164	0.0164
Cheese food .....	125	0.0125

<sup>a</sup> Imported cheeses.

<sup>b</sup> Cheddar.

tyramine was recrystallized once from dilute alcohol and its melting point determined on a hot-stage microscope. A sample of dibenzoyl tyramine was prepared in the same manner from highly purified Eastman Kodak tyramine hydrochloride and the melting point of the derivative taken by the hot-stage microscope. Gale (6) reported that the dibenzoyl tyramine obtained in his studies had a melting point of 171–172° C. (corr.). The derivative from the present cheese and from the known pure tyramine melted at 170–172° C. (corr.) and a melting point of the mixed samples was 169–172° C. (corr.). These data confirm that tyramine was the chemical substance being extracted from the cheese.



## DISCUSSION

It has been possible to show the presence and concentration of tyramine (*p*-hydroxyphenylethylamine) in a large number of ripened types of cheese. As tyramine can be derived from tyrosine by bacterial decarboxylases, a prerequisite of tyramine production in cheese is the presence of free tyrosine. The observations of Dorn and Dahlberg (3) that the white particles in ripened Cheddar cheese actually are made up largely of tyrosine fully satisfies this condition.

The fact that tyramine was found in practically all commercial cheeses examined is not as surprising as the concentrations found. Past concepts that tyramine occurs in normal cheese only in traces, if at all, will have to be revised. A concentration of from 0.08 to 0.12 per cent cannot be considered a trace. In these analyses it was found that 5 out of 25 commercial American Cheddar cheeses fell in the above group. Because of the very unique properties of tyramine, the implications and significance of the presence of such amounts in cheese should pose some very interesting questions for future study and should prove to be a fertile field for investigation.

Compounds other than tyramine and tyrosine, but containing the same characteristic phenolic hydroxyl grouping, if present, also would give a positive Millon test. These compounds would include tyrosol, *p*-hydroxyphenyllactic acid, *p*-hydroxyphenylacetic acid, thyroxine, dopa, phenolphthalein, phenol, salicylic acid and thymol. However, practically all of these compounds can be ruled out either as being insoluble in ether or as never having been reported to be found in cheese. If free phenol were present, it would be removed by the acid ether extraction. Nevertheless, to make certain that tyramine actually was being obtained, a dibenzoyl derivative of it was isolated and purified from cheeses after standard extraction by the method.

The initial 5-hour acid extraction was introduced in the method to remove fat and fatty acids. At the end of this period most of the fat will be extracted, thus ruling out fat as an experimental factor, and no tyramine will be extracted. This was true for all Cheddar cheeses which are extracted at a pH range of 5.0 to 5.8, but in the case of well broken-down cheeses such as Liederkrantz, where the pH was higher, some tyramine was recovered. In the actual analyses these initial recoveries of tyramine were added into the total.

The method as applied to cheese usually produced results on duplicate samples with an experimental error of less than 3 per cent. Results below 50  $\gamma$  per g. produced a larger experimental error. However, as the range of values was from 25  $\gamma$  to more than 2,000  $\gamma$ , this variation was not important.

Several precautions should be stressed. Unless the extraction rate is

very slow at the beginning, a stable sludge or emulsion will occur in the ether of the extraction tube, finally increasing in amount to such a point that it will carry over into the receiver tube. If this happens, a complete re-extraction must be begun. To overcome this emulsification effect it has been found feasible to conduct the extraction for the first 12 hours at a rate of not more than 40 drops per minute. If an emulsion has formed but has not gone through the side arm, the extraction tube may be disengaged and the thimble removed, after which the extraction tube is placed in the warm oil until the emulsion has settled. After slight cooling of the tube, the thimble is replaced, more ether is added, and the extraction process is continued. Other factors which have been observed to aid in forming this emulsion are the presence of green cheese and the extraction of greater amounts of cheese than that recommended here.

The final acid solution in the receiver tube usually is clear. If the solution contains protein particles carried over by the emulsion, usually easily visible, the results will be in error and cannot be used. Also, the ether must be completely boiled off; failure to do this will increase turbidity and will provide erroneous results. A blank should be run on reagents.

#### SUMMARY

A method for separating tyramine from tyrosine and for estimating the concentration of the former substance was applied to cheese. This method involved the use of a continuous ether extractor and the employment of the Millon reagent using a colorimeter.

Twenty-five samples of commercial American Cheddar cheese of different age and history were tested for tyramine. All were found to contain tyramine in varying degrees of concentration. The average for these samples was 384  $\gamma$  per g. or 0.0384 per cent; the highest concentration was 1,199  $\gamma$  per g. or 0.1199 per cent, and the lowest was 25  $\gamma$  per g., or 0.0025 per cent.

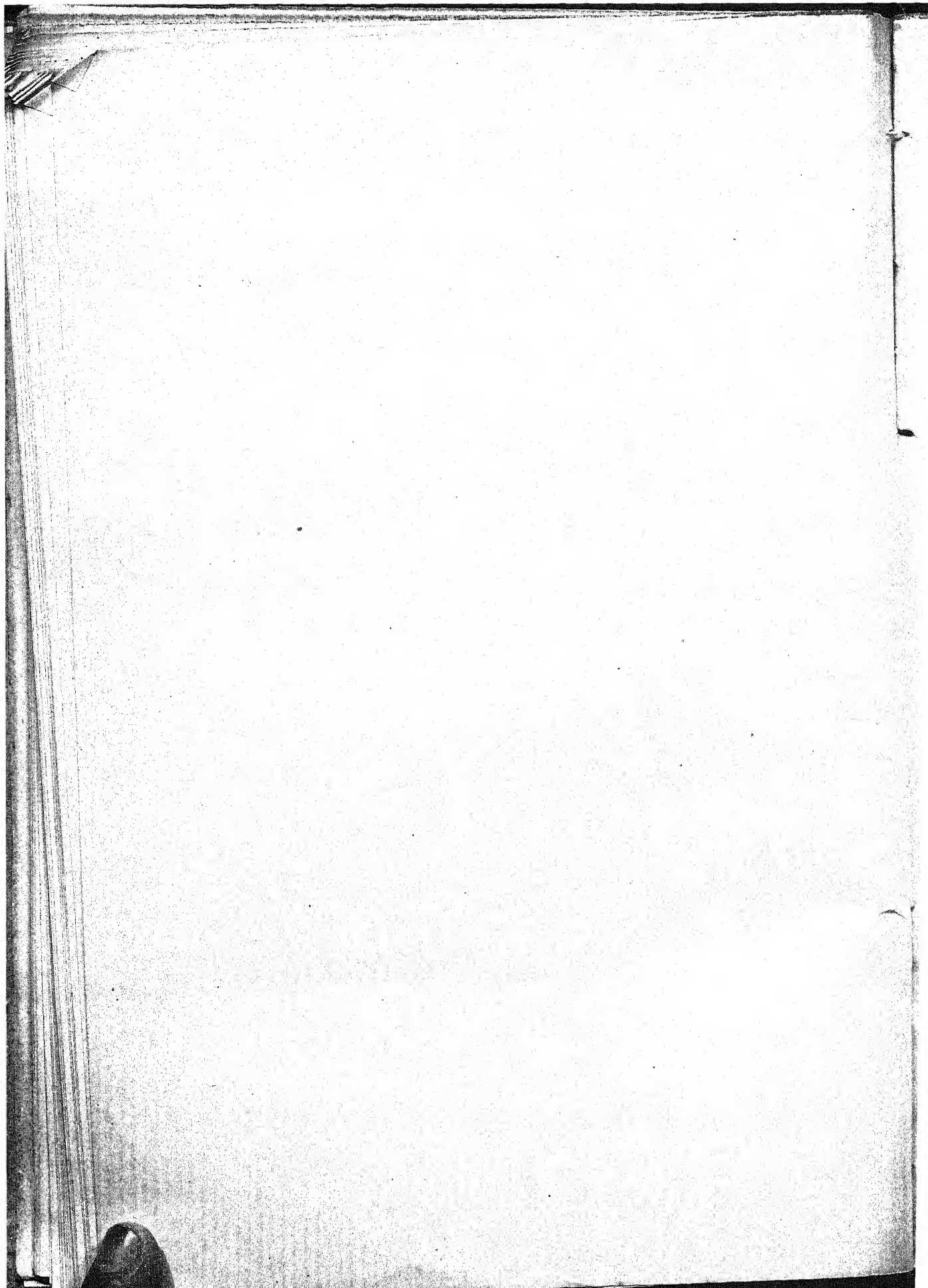
A large number of miscellaneous varieties of ripened types of commercial cheese were tested for tyramine. Again, all samples were found to contain tyramine in varying concentrations. The largest amount was found in a Liederkrantz cheese which had a concentration of 1,683  $\gamma$  per g., or 0.1683 per cent, while the smallest amount was found in a Roquefort cheese with a concentration of 48  $\gamma$  per g. or 0.0048 per cent. The analyses were not sufficiently extensive to establish differences due to cheese variety.

Tyramine was isolated from a sample of Cheddar cheese by this method, purified, and identified as the dibenzoyl derivative.

## REFERENCES

- (1) BELLAMY, W. D., AND GUNSALUS, I. C. Determination of Tyrosine and Tyramine. Mimeographed outline. Unpublished. 1944.
- (2) BELLAMY, W. D., AND GUNSALUS, I. C. Tyrosine Decarboxylation by *Streptococci*; Growth Requirements for Active Cell Production. *J. Bact.*, 48: 191-199. 1944.
- (3) DORN, F. L., AND DAHLBERG, A. C. Identification of the White Particles Found on Ripened Cheddar Cheese. *J. Dairy Sci.*, 25: 31-36. 1942.
- (4) EHRLICH, F., AND LANGE, F. Zur Kenntnis der Biochemie der Käse- reifung. I. Über das Vorkommen von p-Oxyphenyläthylamin in normalen Käse und seine Bildung durch Milchsäurebakterien. *Biochem. Z.*, 63: 156-169. 1914.
- (5) EMERSON, R. L. Über das Auftreten von Oxyphenyläthylamin bei Pankreasver- dauung und über fermentative CO<sub>2</sub>-Abspaltung. *Beitr. Chem. Physiol. u. Path.*, 1: 501-506. 1902.
- (6) GALE, E. F. The Production of Amines by Bacteria. 2. The Production of Tyramine by *Streptococcus faecalis*. *Biochem. J.*, 34: 846-852. 1940.
- (7) HENZE, M. Mikro-bestimmung der Tyramin und Tyrosine. *Z. physiol. Chem.*, 182: 227-240. 1929.
- (8) VAN SLYKE, L. L., AND HART, E. B. The Relation of Carbon Dioxide to Pro- teolysis in the Ripening of Cheddar Cheese. *N. Y. State Agr. Expt. Sta. Bull.* 231. 1903. (Also in *Chem. Centr.*, 11: 133. 1903.)
- (9) WINTERSTEIN, E. Über die Bestandteile des Emmentalerkäses. V. Mitteilung. *Z. physiol. Chem.*, 105: 25-30. 1919.
- (10) WINTERSTEIN, E., AND KÜNG, A. Über das Auftreten von p-Oxyphenyläthylamin in Emmentaler Käse. IV. Mitteilung. Über die Bestandteile des Emmentaler Käses. *Z. physiol. Chem.*, 59: 138-140. 1909.
- (11) WOOLEY, D. W. Improved Extractor for Monoamino Acids. *Ind. Eng. Chem. Anal. Ed.*, 9: 433. 1937.





# THE RELATIONSHIP OF THE AMOUNT OF TYRAMINE AND THE NUMBERS OF *STREPTOCOCCUS FAECALIS* TO THE INTENSITY OF FLAVOR IN AMERICAN CHEDDAR CHEESE<sup>1</sup>

A. C. DAHLBERG AND F. V. KOSIKOWSKY

Department of Dairy Industry, Cornell University, Ithaca, New York

The three previous papers in this series (3, 7, 8) have shown that the flavor of experimental American Cheddar cheese was intensified by *Streptococcus faecalis* starter, that this bacterium grew and survived in large numbers in cheese, and that tyramine, a product of growth of *S. faecalis* in the presence of tyrosine, was found in all varieties of cheese that were analyzed. Problems naturally arose concerning the effect of the presence of *S. faecalis* on the production of tyramine in cheese and any relationship that might exist between the tyramine content of commercial American Cheddar cheese and the intensity of the Cheddar flavor.

## EXPERIMENTAL PROCEDURE

When the cheese from earlier experiments (3) was 5 months old, it was analyzed for tyramine. The flavor intensity of this cheese had been recorded as scored, but the scoring occurred on various days and the samples were not compared together as a lot at any time. Hence, some variation in flavor ratings must be expected due to the personal factor. The obvious relationship of tyramine to flavor intensity prompted further study.

Samples of good commercial Cheddar cheese were selected by several cheese companies to give flavor of varying intensity. These samples came from Wisconsin, Illinois and Missouri. A few samples were selected by one of the authors at factories in New York State. One sample of cheese made at Cornell University from raw milk was used; this sample was 10 years old. After all of the samples were on hand, they were arranged in order of intensity of flavor by three experienced judges. In several instances, the character of the flavor was such that it was difficult to place the cheese exactly. For example, cheese S25 possessed some Swiss cheese flavor which interfered with judgment of the intensity of Cheddar flavor.

Data were obtained on the age of the cheese, and curing temperatures usually were available. The manufacturer also stated whether the cheese had been made from raw or pasteurized milk. The cheese was tested for phosphatase by the method of Sanders and Sager (9), soluble and total

Received for publication December 10, 1947.

<sup>1</sup>This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Lois Phelps, Miss Catherine Verwoert, and Mr. Allan Leventhal for making many of the chemical analyses and to Mr. W. E. Ayres for assisting in the judging of the samples.

protein (2, 10), volatile acids (6), pH, moisture, salt, titratable acidity, total bacterial count by the standard plate method (1), total enterococcus count (13), and *S. faecalis* identified by characteristics given by Sherman (11). The titratable acidity was determined by weighing out 3 g. of cheese, macerating with 10 ml. warm water in a white dish, titrating with 0.1 N alkali with phenolphthalein as indicator, and calculating the results to percentage of lactic acid.

## RESULTS

The experimental cheese made with lactic starter and aged 5 months showed the lowest tyramine content, averaging 38  $\gamma$  per g., and the mildest Cheddar flavor (table 1). Cheese made with both commercial lactic and *S. faecalis* starters developed the highest tyramine content, 786  $\gamma$  per g.,

TABLE 1

*The tyramine content and intensity of flavor of experimental American Cheddar cheese made with commercial lactic and S. faecalis starters, ripened about 5 months*

Cheese no.	Curing temperature	Tyramine	Flavor intensity
	(° F)	( $\gamma$ /g.)	
	Commercial lactic starter		
10464	50	21	medium—
10464	60	87	medium +
10468	50	4	mild
10468	60	40	medium
	Commercial lactic and <i>S. faecalis</i> starters		
10464	50	367	medium +
10464	60	1397	sharp—
10468	50	333	medium
10468	60	1049	medium +
	<i>S. faecalis</i> starter		
10464	50	170	medium
10464	60	830	sharp—
10468	50	122	medium—
10468	60	302	medium +

and the most pronounced Cheddar flavor. The cheese with *S. faecalis* starter alone contained 356  $\gamma$  of tyramine per g. Very little tyramine was produced in cheese made from pasteurized market milk with ordinary lactic starter, whereas *S. faecalis* starter produced large amounts of tyramine. It may be observed also that there is an associated action between the two starters, which results in the production of more tyramine and slightly more flavor than obtained by *S. faecalis* alone. The acceleration of tyramine production at 60° F. as compared with 50° F. may be noted, and will be considered further in a subsequent paper.



Some variation in the exact relationship was noted in individual samples, but the intensity of the flavor of Cheddar cheese increased as the tyramine content increased (fig. 1). The trend line, drawn empirically, not only shows the trend but also, with one exception, divides the figure so that all samples of cheese above the line were cured at 50° F. and those below the line at 60° F. This observation means that the rate of flavor

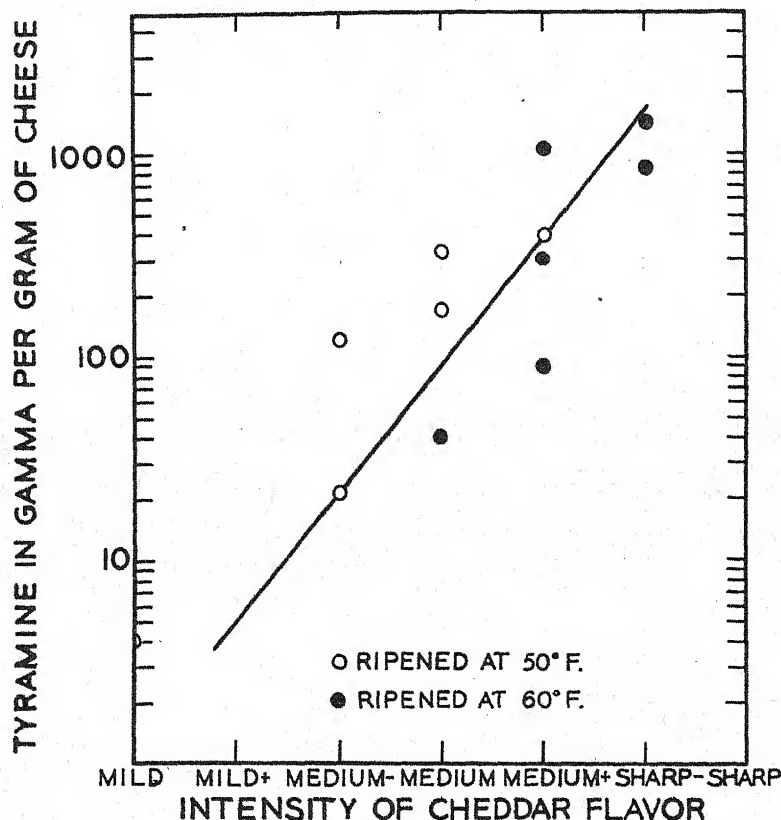


FIG. 1. The relationship between tyramine content and intensity of flavor of experimental Cheddar cheese made with commercial lactic and *S. faecalis* starters. (Ripened about 5 months.)

development at 60° F. was relatively more rapid than the increase in tyramine when compared with the rate of increases at 50° F.

The data on the 24 samples of commercial Cheddar cheese and one sample of old cheese made at Cornell University are presented in tables 2 and 3. The cheeses were grouped into units of five on the basis of flavor intensities; thus numbers 1 to 5 included the five sharpest flavored cheese in the experiment (table 4). There were fifteen raw milk cheese, six made from pasteurized milk and four were underpasteurized (table 2).

TABLE 2  
The age, acidity, composition and flavor intensity of commercial American Cheddar cheese made from raw and pasteurized milk

Cheese no.	Age in months	Pasteurization		pH	Titratable acidity as % lactic	Water (%)	Salt (%)	Order of flavor intensity
		Mfg. report	Phosphatase test <sup>a</sup> (units/0.25 g.)					
O 22	120	raw	40	5.70	4.87	37.5	2.0	1
O 21	36	raw	40	5.42	3.52	31.3	1.7	2
A 4	30	raw	40	5.49	3.36	29.8	1.7	3
M 20	24	raw	30	5.61	2.86	35.0	2.0	4
K 13	10.5 <sup>a</sup>	raw	40	5.35	2.87	34.5	1.4	5
K 12	9.5	raw	40	5.35	2.80	34.2	1.4	6
S 25	33	raw	30	5.35	3.42	37.5	2.0	7
St 15	8.5	raw	35	5.23	2.95	34.7	1.5	8
A 5	10.5	raw	40	5.13	2.82	34.1	2.0	9
K 3	11.5	past.	1	5.22	2.89	37.0	1.6	10
St 14	3	raw	40	5.32	2.71	35.9	1.6	11
K 11	8	raw	40	5.46	2.21	36.6	1.2	12
LM 9	12.5	past.	9	5.22	2.57	33.4	1.9	13
K 2	4.5	past.	2	5.15	2.68	36.1	1.5	14
M 18	2.5	raw	40	5.04	2.31	38.1	1.4	15
M 17	2.5	past.	3	5.15	2.26	37.3	1.5	16
CM 24	2.5	?	6	5.14	2.03	37.2	1.3	17
CM 23	2.5	?	5	5.10	2.18	37.5	2.0	18
LM 8	12.5	past.	2	5.27	2.41	34.3	1.8	19
K 19	2.5	raw	40	5.06	2.42	39.0	1.5	20
LM 7	14.5	past.	3	5.60	2.09	32.4	1.9	21
K 1	2.5	past.	3	5.03	2.16	37.0	1.5	22
K 10	1.5	raw	40	5.26	1.92	35.7	1.5	23
M 16	1.5	raw	40	5.13	1.91	39.0	1.6	24
A 6	6	past.	5	5.32	1.71	36.0	1.4	25

<sup>a</sup> Values of 5 or greater indicate raw milk or contamination with raw milk.

TABLE 3

The flavor intensity, volatile acidity, soluble protein, and tyramine content and *Streptococcus faecalis* in commercial American Cheddar cheese made from raw and pasteurized milk

Order of intensity	Flavor		Volatile acids (ml. 0.1 N/100 g.)	Protein		Tyramine ( $\mu$ /g.)	Bacterial counts (in thousands)		
	Intensity grade			Soluble (%)	Soluble % of total		Standard plate count (per g.)	Enterococci (per g.)	<i>S. faecalis</i> (per g.)
1	sharp	++	173.3	12.6	57.3	2330	3,000	<100	<10
2	sharp	++	48.8	11.7	45.2	966	56,000	22,000	1,000
3	sharp	++	34.1	10.6	40.8	1199	17,000	4,000	80
4	sharp	++	24.8	11.7	42.9	1147	30,000	18,000	17,000
5	sharp	—	37.8	8.3	31.9	847	380,000	80,000	56,000
6	sharp	—	37.4	8.0	31.4	814	200,000	91,000	82,000
7	sharp	—	39.5	12.0	44.0	460	3,000	900	300
8	medium	+	41.3	7.7	32.1	746	129,000	28,000	3,000
9	medium	+	17.5	7.4	38.5	406	125,000	39,000	8,000
10	medium	+	39.3	7.5	29.7	566	285,000	53,000	3,000
11	medium	—	37.1	5.1	20.3	377	535,000	265,000	159,000
12	medium	—	34.1	6.6	25.1	233	623,000	128,000	58,000
13	medium	—	20.9	7.2	30.0	112	7,000	<100	<10
14	medium	—	40.9	6.4	25.2	177	20,000	13,000	1,000
15	medium	—	35.2	4.7	20.8	230	106,000	98,000	10,000
16	mild	+	32.2	5.1	20.8	147	55,000	29,000	<1,000
17	mild	+	22.6	4.3	19.6	123	86,000	27,000	5,000
18	mild	+	18.7	4.5	19.9	134	145,000	52,000	3,000
19	mild	—	11.9	7.1	29.5	55	10,000	<100	<100
20	mild	—	26.7	3.6	24.6	119	450,000	73,000	18,000
21	mild	—	15.5	7.7	32.1	58	40,000	<100	<10
22	mild	—	19.9	3.9	16.1	30	6,000	3,000	<100
23	mild	—	24.9	3.1	18.5	59	50,000	21,000	19,000
24	mild	—	17.6	3.0	13.3	99	205,000	90,000	31,000
25	mild	—	16.2	4.5	18.8	25	1,000	500	<20



Although cheese must be aged to develop flavor, the relationship between age and intensity of flavor was not very exact. The cheese that was 120 months old was highest in flavor, but a 6-month-old cheese was the mildest in flavor, being less cured than some cheese only 1.5 months old (table 2). The summarized data show that the ten strongest flavored cheese were oldest, but the next 15 cheese were rather uniform in age (table 4).

It is known that the pH of cheese increases with age. Apparently variations in individual cheese exceeded the effect of aging upon pH or any possible relationship of intensity of flavor to pH (tables 2 and 4). The average pH was near 5.2, the highest flavored group averaged 5.5, and the pH of all samples varied from 5.03 to 5.70. The titratable acidity varied from 1.71 for the mildest flavored cheese to 4.87 for that with the strongest flavor.

TABLE 4

*The age, acidity, soluble protein, and tyramine content of commercial American Cheddar cheese grouped in units of 5 on the basis of flavor intensity*

Order of flavor intensity	Age in months	pH	Titra- table acidity	Volatile acids	% soluble protein	% of total protein that is soluble	Tyra- mine
				(ml. 0.1 N acid/100 g.)			( $\gamma$ /g.)
1-5	44.1	5.51	3.49	63.7	11.0	43.6	1258
6-10	14.6	5.25	2.97	35.0	8.5	33.1	598
11-15	6.1	5.24	2.49	33.6	6.0	24.3	226
16-20	4.6	5.14	2.26	22.4	4.9	22.9	115
21-25	5.2	5.27	1.96	18.8	4.4	19.8	54

The higher the titratable acidity of cheese, the more intense the cheese flavor (table 4); even the results on individual samples of cheese were noticeably consistent. There was no striking exception to this general relationship (table 2).

No significance was attached to the moisture contents, which varied from 29.8 to 39.0 per cent, and to the salt contents, which varied from 1.2 to 2.0 per cent.

Although previous studies by the authors (3) had established no definite relationship between the intensity of flavor of Cheddar cheese and volatile acidity or water soluble protein, this work was repeated in this study. In a general way, in average grouped data, the volatile acidity and soluble protein values increase with increased flavor (table 4), but this relationship does not hold for individual samples (table 3). For example, the cheese with the fourth sharpest flavor and the cheese that was twenty-third in flavor intensity both had volatile acids per 100 g. equivalent to 24.9 ml. 0.1 N alkali. The volatile acidity ranged from 16.2 to 173.3. The soluble

protein varied from 3.0 to 12.6 per cent, and the percentage of the total protein that was water soluble varied from 13.3 to 57.3 per cent, with noticeable exceptions to a definite correlation to flavor intensity in individual samples.

The data show a definite relationship of increased tyramine content and increased flavor. In the individual samples of cheese, the strongest flavored Cheddar cheese contained 2,330  $\gamma$  of tyramine per g., and the twenty-fifth cheese in flavor intensity, the mildest of all flavors, had only

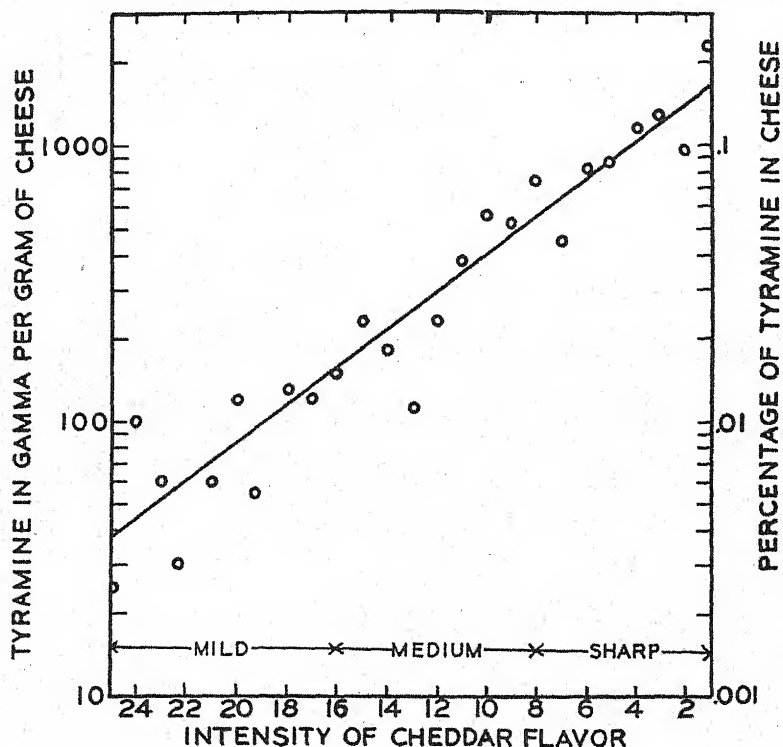


FIG. 2. The relationship between tyramine content and intensity of flavor of commercial American Cheddar cheese.

25  $\gamma$  of tyramine (table 3). The tyramine ratio of these cheeses was 93 to 1. A close relationship between the tyramine content and intensity of flavor of individual samples of cheese is shown in figure 2. When one considers that there was no control over the quality of milk, method of manufacture, starter, time and temperature of ripening, and other factors of possible significance, the relationship is exceptionally good. The relationship between the tyramine content and flavor intensity was a direct semilogarithmic one. On the basis of groups of cheese as units of five in order of flavor intensity, the gamma of tyramine per gram was doubled for each group

(table 4). Tyramine added to fresh cheese curd did not give Cheddar cheese flavor and it did not aid the development of flavor during ripening.

The total bacterial counts on the cheese were made with standard tryptone-glucose-extract-milk agar, but the plates were incubated at 32° C. for 4 days to obtain maximum counts. The total bacterial counts per gram of cheese ranged from 1 million to 623 million. Counts also were made with the penicillin-azide medium of White and Sherman (13) and these counts, considered to be chiefly enterococci, ranged from less than 100,000 to 265 million. From each plate count for enterococci, 20 colonies were isolated and the number of *S. faecalis* determined, except for the possibility of confusion with *Streptococcus zymogenes*, which is not common in milk. The *S. faecalis* count was calculated from the proportion of the colonies which proved to be *S. faecalis*. The *S. faecalis* count varied from less than 20,000 to 159 million per g., and *S. faecalis* actually was isolated from 18 of the 25 samples of cheese. There appeared to be no correlation between bacterial counts and flavor intensity, and this observation was expected and in accordance with several previous publications.

#### DISCUSSION

It has been shown that there is a direct relationship between the tyramine content of American Cheddar cheese and the intensity of its flavor. The correlation was better for good commercial cheese selected at random than for experimental cheese made from good pasteurized market milk with special cultures and cured at different temperatures. Tyramine added to cheese curd did not give the Cheddar flavor, so tyramine is not the flavor compound. The amount of tyramine indicated the extent of activity of *S. faecalis* and probably of other bacteria, such as certain strains of the lactobacilli, which possibly may produce tyramine (4, 5). The numbers of these bacteria may increase in the early stages of cheese ripening and then decrease, so that these numbers at any one time may not be too significant.

The growth of *S. faecalis* in cheese produces Cheddar flavor, but this is only one source of flavor. That other factors are involved is indicated by the development of some Cheddar flavor in cheese with amounts of tyramine too small to indicate much growth of *S. faecalis*, and by the more rapid production of flavor than of tyramine at 60° F. when compared to 50° F. Flavor with low tyramine content always was flat, irrespective of age of the cheese.

*S. faecalis* and lactobacilli should be present in all raw milk Cheddar cheese, as these bacteria occur universally in raw milk. Both types of bacteria are involved in cheese ripening. *S. faecalis* is thermotolerant, although survival numbers are not great, and about half of the lactobacilli



are not destroyed by pasteurization (12). Hence, pasteurized milk cheese cures slowly, as does cheese made from very low-count raw milk. The survival of *S. faecalis* and lactobacilli during pasteurization must be an important factor in present day curing of pasteurized milk cheese. The small numbers of these bacteria are increased by warmer curing temperatures.

It was reported in the first paper in this series (3) that cheese made with lactic and *S. faecalis* starters developed more flavor than cheese made with either starter alone, and that the flavor with lactic starter only was especially mild. This observation was related directly to the production of tyramine. Lactic and *S. faecalis* starters together produced more tyramine in cheese than did *S. faecalis* starter alone, even though the numbers of *S. faecalis* bacteria were greater in the cheese in which this culture alone was used, probably due to the larger inoculation in the milk. The lactic starter induced higher flavor development in cheese by its symbiotic action with *S. faecalis*.

#### SUMMARY

Experimental American Cheddar cheese made with commercial lactic starter from pasteurized milk developed low amounts of tyramine, 4 to 87  $\gamma$  per g., and flavor of mild to medium intensity in 5 months of curing. The combination of lactic and *Streptococcus faecalis* starters in cheese produced the largest amounts of tyramine, 333 to 1,397  $\gamma$  per gram, and flavor of medium to sharp intensity. *S. faecalis* starter alone in cheese produced tyramine and flavor between these two extremes.

In commercial American Cheddar cheese made from raw and pasteurized milk, cured for varying periods, there was a direct semilogarithmic relationship between tyramine content (25 to 2,330  $\gamma$  per g.) and the intensity of flavor. Of the 25 cheese samples, 18 gave plate counts on the special medium of over 80,000 *S. faecalis* per g., with the high count of 159 million. The *S. faecalis* bacteria produced the tyramine, although other bacteria may contribute.

Tyramine was not the Cheddar-flavor compound, but served as a means of measuring bacterial activity that accentuated flavor production. The activity of bacteria producing tyramine did not account for all cheese flavor.

The increase in titratable acidity was related directly to cheese flavor intensity, and this relationship, even though subject to considerable variation, was too close in individual samples to be accidental. In a general way, the increase in volatile fatty acids and water soluble nitrogen was related to flavor intensity, but variations in individual samples prevented a definite correlation and also established that these changes were incidental to flavor development.

## REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 9th Ed. New York, N. Y. 1946.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 5th Ed. Washington, D. C. 1940.
- (3) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Development of Flavor in American Cheddar Cheese Made from Pasteurized Milk with *Streptococcus faecalis* Starter. J. Dairy Sci., 31: 275-284. 1948.
- (4) GALE, E. F. Chapter on The Bacterial Amino Acid Decarboxylases in Advances in Enzymology, 6: 1-32. Edited by F. F. Nord. Interscience Publishers, Inc., New York, N. Y. 1946.
- (5) HANKE, M. T., AND KOESSLER, K. K. Studies on Proteinogenous Amines. XVII. The Faculty of Normal Intestinal Bacteria to Form Toxic Amines. J. Biol. Chem., 59: 835-853. 1924.
- (6) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. J. Dairy Sci., 29: 861-871. 1946.
- (7) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Growth and Survival of *Streptococcus faecalis* in Pasteurized-milk American Cheddar Cheese. J. Dairy Sci., 31: 285-292.
- (8) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Tyramine Content of Cheese. J. Dairy Sci., 31: 293-303. 1948.
- (9) SANDERS, G. P., AND SAGER, O. S. Modification of the Phosphatase Test as Applied to Cheddar Cheese and Application of the Test to Fluid Milk. J. Dairy Sci., 29: 739-749. 1946. (Also J. Assoc. Offic. Agr. Chemists, 28: 656-675. 1945.)
- (10) SHARP, P. F., private communication, about 1940, as reported by Dahlberg, A. C., and Kosikowsky, F. V. The Flavor, Volatile Acidity, and Soluble Protein of Cheddar and Other Cheese. J. Dairy Sci., 30: 165-174. 1947.
- (11) SHERMAN, J. M. The Streptococci. Bact. Revs., 1: 3-97. 1937.
- (12) SLATTER, W. L., AND HALVORSON, H. O. The Heat Resistance of Lactobacilli Found in American Cheddar Cheese. J. Dairy Sci., 30: 231-243. 1947.
- (13) WHITE, J. C., AND SHERMAN, J. M. Occurrence of Enterococci in Milk. J. Bact., 48: 262. 1944.

## DETERMINATION OF VITAMIN A IN MILK<sup>1</sup>

MELVIN HOCHBERG

*Vitamin Products Laboratory, Nopco Chemical Company, Harrison, New Jersey*

Milk is an important natural source of dietary vitamin A. Recent interest in the enrichment of milk has emphasized the need for a simple and reliable method of assay for control purposes. Attempts to determine preformed vitamin A in milk in this laboratory by several methods found in the literature have been unsuccessful. These procedures, although reliable for other types of products, gave low recoveries (approximately 80 per cent of the theoretical) of the added vitamin. In these tests the milk was enriched with a vitamin A emulsion similar in composition to milk itself.<sup>2</sup> The low values may be attributed to a combination of causes, including destruction of the vitamin during hot saponification, inadequate cold saponification, loss due to adsorption on the milk protein, inefficient extraction, or failure to take into account the effect of inhibitors in the antimony trichloride reaction.

In the present study, vitamin A in milk is determined quantitatively by a method which involves cold saponification with potassium hydroxide, extraction of the vitamin with diethyl ether, evaporation of the solvent, and solution of the residue in chloroform.<sup>3</sup> The vitamin then is allowed to react with antimony trichloride and the resulting blue color measured in an Evelyn photoelectric colorimeter. The effect of compounds which inhibit the color formation is evaluated by means of an internal standard.

The importance of adequate, cold saponification in the analysis of milk for vitamin A has been recognized by other investigators (1). The use of the internal standard has been described (5) and has received considerable attention in the development of analytical procedures for the determination of vitamin A in enriched margarine.

### METHOD

#### *Reagents*

*Aldehyde-free alcohol.* Reflux 1 l. of 95 per cent ethyl alcohol with 10 g. of potassium hydroxide on a steam bath for 5 hours and then cool. Add 0.5 g. granulated aluminum and distill on a steam bath. Prepare fresh each month.

Received for publication January 29, 1948.

<sup>1</sup> Contribution from the Vitex Department, Vitamin Division, Nopco Chemical Company, Harrison, New Jersey.

<sup>2</sup> The concentrate used for enrichment consisted of a butter oil solution of vitamin A, homogenized with water and skim milk solids.

<sup>3</sup> The saponification, extraction, evaporation, and washing steps are essentially those employed in the laboratory of the Wisconsin Alumni Research Foundation. The details of the method were kindly supplied by Dr. Carl H. Krieger.



*Sixty per cent potassium hydroxide solution.* Dissolve 60 g. of potassium hydroxide in 40 ml. of distilled water.

*Antimony trichloride reagent.* Grind 250 g. of antimony trichloride with 250 g. of anhydrous sodium sulfate. Suspend the mixture in 1,000 ml. of freshly redistilled chloroform, stir mechanically for 15 minutes, filter through rapid paper, and store in the dark in a glass-stoppered amber bottle. The reagent is stable for at least 1 month. If turbid, filter again immediately before use.

*Saturated sodium chloride solution.* Suspend 800 g. of sodium chloride in 2,000 ml. of water, stir mechanically for 15 minutes and allow to stand overnight to assure saturation.

*Ethyl ether.* Distill U.S.P. ethyl ether freshly each day.

*Chloroform.* Distill C.P. grade chloroform freshly each day discarding the first 10 per cent of the distillate.<sup>4</sup>

*Vitamin A standard.* Dissolve 100 mg. of U.S.P. vitamin A Reference Standard (10,000 U.S.P. units per g.) in 100 ml. of freshly redistilled chloroform. This solution contains 10 U.S.P. units of vitamin A per ml. and is prepared fresh each day.

*Acetic anhydride.* Use C.P. grade.

#### *Special Apparatus*

*Separatory funnels.* Three 500-ml. pear-shaped separatory funnels are required.

*Evelyn photoelectric colorimeter (macro) with matched colorimeter tubes.* This instrument is manufactured by the Rubicon Company, Philadelphia, Pennsylvania.

*Rapid delivery pipette.* This may be prepared by cutting the tip from a 10-ml. volumetric pipette, leaving an opening about 2 mm. in diameter. The pipette need not be recalibrated.

#### *Procedure*

To 100 ml. of milk in an amber Erlenmeyer flask are added 50 ml. of aldehyde-free alcohol and 10 ml. of 60 per cent potassium hydroxide. The suspension is mixed and allowed to stand overnight in a dark cabinet. In the morning the sample is transferred to a 500-ml. amber separatory funnel. The flask is washed consecutively with 40-, 40-, and 20-ml. portions of distilled water and once with 100 ml. of redistilled ether. The washings are added to the separatory funnel. The latter is shaken thoroughly and then allowed to stand until a sharp separation of the phases is

<sup>4</sup>Occasionally samples of chloroform are obtained which are unsatisfactory as solvents for vitamin A. The vitamin standard solution in chloroform should show less than 5% decomposition when stored for 24 hours in the dark.

observed. The lower layer is drawn off into a second separatory funnel. This is shaken with 75 ml. of ether. After the phases have separated, the lower (aqueous) layer is drawn off into a third separatory funnel. The ether layer is added to the extract in the first funnel, along with a 25-ml. ether wash. The aqueous phase is re-extracted twice more with 75- and 50-ml. portions of ether, respectively. The ether phases all are combined in the first separatory funnel and are washed four times with 100-ml. portions of distilled water. To prevent the formation of emulsions, the first two washings merely are poured through the ether, without shaking, and then drawn off. The funnel is shaken gently during the third washing and vigorously during the fourth. Sufficient time for complete separation of the phases must be allowed at all times. Finally, the ether extract is washed twice with 75-ml. portions of saturated sodium chloride solution, shaking vigorously each time. The last salt solution is separated sharply from the ether phase.

The ether extract is transferred to a 500-ml. amber distilling flask containing two or three glass beads. The separatory funnel is rinsed with 25 ml. of redistilled ether and the wash added to the flask. The ether is removed by distillation on a steam bath until approximately 5 ml. remain in the flask. The remainder is evaporated off at room temperature with the aid of a stream of carbon dioxide. While still under the inert atmosphere, the vitamin A in the residue is dissolved immediately in sufficient chloroform to produce a concentration of approximately 10 U.S.P. units per ml.

Into a series of labeled Evelyn colorimeter tubes in a wooden rack are pipetted 2 ml. of chloroform solvent (tube *A*), 1 ml. of chloroform extract of sample + 1 ml. of chloroform solvent (tube *B*), and 1 ml. of chloroform extract of sample + 1 ml. of chloroform solution of vitamin A standard (tube *C*).

To tube *A*, one drop of acetic anhydride is added, followed by 10 ml. of antimony trichloride reagent, the latter from the rapid delivery pipette.<sup>5</sup> The colorimeter, containing a 620 m $\mu$  filter, then is set at 100 per cent transmission with the solution in tube *A*. The tube is removed and the "center setting" noted. The latter is employed to reset the instrument before each subsequent reading.

Tube *B* is placed in the instrument, one drop of acetic anhydride is added, and 10 ml. of antimony trichloride reagent are added rapidly. The galvanometer needle first fluctuates rapidly, "pauses" for a second or two, then drifts slowly as the blue color fades. The per cent transmission at the pause point is recorded. Tube *C* is measured similarly.

<sup>5</sup> Because antimony trichloride solution is extremely sensitive to moisture and is corrosive, it must be pipetted with a rubber bulb, not by mouth.

Because of difficulty in observing the pause point and in order to obtain reliable results, tubes *B* and *C* are set up and measured in triplicate, and the average per cent transmission of each determined. The latter (*G*) is converted to photometric density ( $L = 2 - \log G$ ), employing the chart provided with the instrument. The vitamin A content of the milk sample is calculated employing the formula:

$$\frac{L_B}{L_v - L_B} \times 10 \times \frac{V}{100} \times 946 = \text{U.S.P. units of vitamin per quart.}$$

(*V* = cc. of chloroform solution of unsaponifiable extract)

#### EXPERIMENTAL RESULTS AND DISCUSSION

The colorimetric method as described by Oser *et al.* (5) has been employed successfully in these and other laboratories for the determination

TABLE 1

*Recovery tests of vitamin A added to milk, employing previously published procedures (1, 5)<sup>a</sup>*

Procedure	Milk sample	Vitamin A added	Total vitamin A found	Vitamin A recovered	
		(USP units per qt.)			(%)
Boyer <i>et al.</i> (1)	Jan. milk, homogenized	0	960	.....	.....
		2000	2560	1600	80
		4000	3970	3010	75
		6000	6190	5230	87
	Oct. milk, cream-line	0	1020	.....	.....
		4000	4270	3250	81
Oser <i>et al.</i> (5)	Oct. milk, homogenized	0	1510	.....	.....
		4000	4800	3290	83
	Oct. milk, homogenized	0	1420	.....	.....
		4000	4700	3280	82
	Oct. milk, cream-line	0	1320	.....	.....
		4000	4580	3260	82
	Oct. milk, homogenized	0	1200	.....	.....
		4000	4400	3200	80

<sup>a</sup> For each recovery test, the vitamin was added directly to the milk immediately before the analysis and the enriched sample was carried through the entire determination. The concentrate employed for enrichment consisted of a solution of vitamin A esters in butter oil, homogenized with water and skim milk solids.

of vitamin A in a wide variety of food products. Boyer *et al.* (1) have applied the antimony trichloride reaction to the analysis of milk. The recent interest in the enrichment of milk with vitamin A prompted an examination of the reliability of these procedures. The results of these tests are presented in table 1. Milk samples were enriched with vitamin A at the levels indicated, employing a concentrate similar in composition to milk itself. This concentrate consisted of a solution of vitamin A



esters in butter oil, homogenized with water and skim milk solids, and contained 30,000 U.S.P. units per ml. The blank and enriched samples were analyzed immediately according to the published procedures. Approximately 80 per cent of the added vitamin was recovered in each case. Because of the physical nature of milk, the complete extraction of its fat-soluble constituents is particularly difficult. Therefore, it is not surprising that the determination of vitamin A in milk requires a special technique.

Similar recovery tests, employing the procedure described in the present

TABLE 2  
*Recovery tests of vitamin A added to milk employing present procedure<sup>a</sup>*

Milk sample	Vitamin A added	Total vitamin A found	Vitamin A recovered	
	(USP units per qt.)			(%)
<i>A</i>	0	1310	.....	.....
	2000	3270	1960	98
Oct. milk,	4000	5500	4190	105
homogenized	6000	6970	5660	94
<i>B</i>				
Oct. milk,	0	1200	.....	.....
cream-line	4000	5250	4050	101
<i>C</i>				
Oct. milk,	0	1070	.....	.....
cream-line	4000	4970	3900	98
<i>D</i>				
Jan. milk,	0	830	.....	.....
homogenized	4000	4830	4000	100
<i>E</i>				
Jan. milk,	0	920	.....	.....
cream-line	4000	4950	4030	101

<sup>a</sup> For each recovery test, the vitamin was added directly to the milk immediately before the analysis and the enriched sample was carried through the entire determination. The concentrate employed for enrichment consisted of a solution of vitamin A esters in butter oil, homogenized with water and skim milk solids.

paper, are reported in table 2. Recovery values ranged from 94 to 105 per cent, with an average of 99.6 per cent.

The use of an internal standard in the determination of vitamin A decreases the precision of the analysis. Therefore, some investigators have employed a reference curve, obtained with pure solutions, to calculate their results or, since the reaction obeys Beer's law, an external standard of 10 U.S.P. units. The data employed in calculating the results reported in table 2 demonstrate the importance of the internal standard or increment. These data are presented in table 3. In a given test extract, the photometric density has been found to be proportional to the vitamin A content. However, biological materials contain substances which inhibit

the reaction, and the constant of proportionality is not the same in all extracts. Thus in sample A, the increments due to 10 units of added vitamin A were 0.184, 0.174, 0.173 and 0.180, respectively, in the four solutions tested. Employing the same reagents at the same time, 10 units of vitamin A in pure chloroform gave a photometric density of 0.201. Thus, the use of a reference curve in calculating the vitamin A content of the above samples would give values erroneously low by approximately 13 per cent. Samples B and C likewise required the internal standard. On the other hand, either method of calculation could be employed with D and E. The

TABLE 3  
*Importance of the internal standard*

Milk sample	Vitamin A added (USP units per qt.)	Photometric density			
		Tube B	Tube C	Internal standard <sup>a</sup>	External standard <sup>b</sup>
A	0	0.102	0.286	0.184	0.201
	2000	0.240	0.414	0.174	0.201
	4000	0.201	0.374	0.173	0.201
	6000	0.177	0.357	0.180	0.201
B	0	0.047	0.233	0.186	0.199
	4000	0.184	0.362	0.178	0.199
C	0	0.043	0.233	0.190	0.204
	4000	0.182	0.364	0.182	0.204
D	0	0.036	0.240	0.204	0.204
	4000	0.189	0.387	0.198	0.204
E	0	0.039	0.238	0.199	0.204
	4000	0.184	0.387	0.203	0.204

<sup>a</sup> Photometric density due to the reaction of antimony trichloride with 10 USP units of vitamin A added to the chloroform solution of the unsaponifiable extract of the milk sample.

<sup>b</sup> Photometric density due to the reaction of antimony trichloride with 10 USP units of vitamin A in chloroform.

importance of the internal standard also has been established in the determination of niacin (4) and pyridoxine (3).

If the total vitamin A content of unenriched milk is of interest,  $\beta$ -carotene should be determined in the extract by carefully evaporating off the ethyl ether, taking up the residue in petroleum ether, and fractionating the pigments with diacetone alcohol (2). As much as one-fourth of the total vitamin A potency of unenriched milk is due to carotene, although in the case of summer milk from Guernsey cows, almost 50 per cent may be present as the provitamin (1). In milk enriched with preformed vitamin A, these proportions are considerably smaller.

Though carotene reacts with antimony trichloride to form a blue pigment, this does not interfere appreciably in the determination of the preformed vitamin because of differences in the rates and sensitivities of the two reactions. The photometric density at 620  $m\mu$  due to one U.S.P. unit of  $\beta$ -carotene is only one-twelfth that due to one U.S.P. unit of preformed vitamin A 4 seconds after the addition of antimony trichloride reagent (5).

#### SUMMARY

1. Vitamin A in milk was determined quantitatively by cold saponification with potassium hydroxide, extraction with diethyl ether, evaporation of the solvent, and solution of the residue in chloroform. The blue color formed by reaction with antimony trichloride was measured in a photoelectric colorimeter.

2. Theoretical recoveries of added vitamin A were obtained. The concentrate employed was a solution of vitamin A in butter oil, homogenized with water and skim milk solids. Two other procedures for the determination of vitamin A gave recoveries of only 80 per cent.

3. Colorimetric evaluation of the vitamin A content of the final extract included the use of an internal standard. Calculations based upon a reference curve obtained in pure solutions gave low recovery values in some samples.

#### REFERENCES

- (1) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. *Ind. Eng. Chem., Anal. Ed.*, **16**: 101-102. 1944.
- (2) HEGSTED, D. M., PORTER, J. W., AND PETERSON, W. H. Determination of Carotene in Silage. *Ind. Eng. Chem., Anal. Ed.*, **11**: 256-258. 1939.
- (3) HOCHBERG, M., MELNICK, D., AND OSER, B. L. Chemical Determination of Pyridoxine: Reactions in Pure Systems. *J. Biol. Chem.*, **155**: 109-117. 1944.
- (4) MELNICK, D. Collaborative Study of the Applicability of Microbiological and Chemical Methods to the Determination of Niacin in Cereal Products. *Cereal Chem.*, **19**: 553-567. 1942.
- (5) OSER, B. L., MELNICK, D., AND PADER, M. Estimation of Vitamin A in Food Products. *Ind. Eng. Chem., Anal. Ed.*, **15**: 724-729. 1943.





# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

MAY, 1948

NUMBER 5

## THE EFFECT OF CLIPPING THE UDDERS OF COWS ON THE QUALITY OF MILK

E. W. HIRD, TULY REITER, K. G. WECKEL, AND N. N. ALLEN

*University of Wisconsin, Madison*

The effect of clipping of udders of cows upon the quality of milk has been the subject of original study of but a few investigators (2, 3). The information available, though brief, indicates benefit from the practice. Among milk producers and quality supervisors there seems to be varied opinion as to the benefit of the clipping of udders. In view of the increasing emphasis on the methods for obtaining milk of good quality, a study was made of the effect of clipping of udders and adjacent areas on certain quality properties of milk.

### EXPERIMENTAL PROCEDURE

*Handling of Cows.* The experiment was divided into four periods as follows: (a) Control period, milking by machine. November 20 to December 2, 1946. (b) Second period, milking by machine. December 3, 1946, to January 30, 1947. (c) Third period, milking by hand. February 6 to March 26, 1947. (d) Fourth period, milking by machine. March 26 to April 4, 1947. In the control period, prior to clipping any of the animals, the numbers of bacteria in the milk of the individual cows milked by machine were determined. For the second period, alternate cows, as they stood in line, were clipped. The animals were reclipped during the third period. The clipped area was posterior to a line from the pinbones to the navel, including thighs, flanks, and udder, and the tail except for the switch. The area clipped is illustrated in figure 1. As they freshened and were introduced in the milking line, alternate cows were clipped.

The conditions in the barn were comparable to those usually found in a city fluid milk area. Wood shavings were used liberally for bedding, but not in excess. The cows were groomed once daily, usually during the morning but not immediately before the milkings. The night's accumulation of manure was in the gutters at the time of the morning milking. The cows seldom were soiled at milking time to a degree greater than that

Received for publication August 15, 1947.

<sup>1</sup> Project supported by grant from Sunbeam Corporation, Chicago, Ill.

of having loose bedding and dirt clinging to the body. The herd was kept indoors without access to an exercise yard.

*Treatment of milking utensils.* All utensils used in this experiment were new. Prior to each period of use, four disassembled milking machine units, excluding the pails, were sterilized in a steam autoclave. The stainless steel machine pails and milk cans were jet steamed for a period of 3 minutes, then filled with a solution of 200 p.p.m. available chlorine for 1 hour, drained, and covered with parchment paper. The units then were assembled and immersed in a solution containing 200 p.p.m. of chlorine. Two gallons of sterile distilled water added in three portions was used to rinse the chlorine from one of the machine pails chosen at random. The

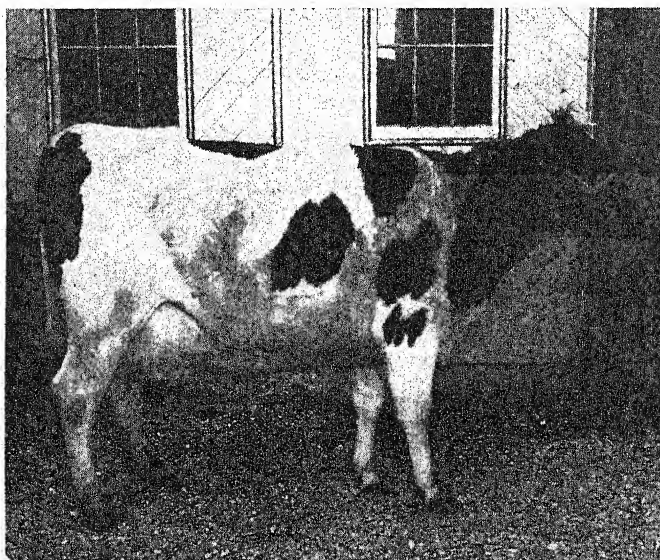


FIG. 1. One of the cows used in the experiment, showing the area clipped.

machine head then was adjusted and another 2 gallons of sterile distilled water drawn through the teat cup assembly and into the pail. The average bacteria count per ml. of the rinse water was ascertained to be 2 in the evening and 1 in the morning tests.

Just before milking, the udder of each cow was wiped with an individual cloth previously immersed in a warm solution of 200 p.p.m. available chlorine. The cows were fore-milked into a strip cup, and this milk was discarded. The sterilized machine unit was attached with special care to avoid contamination. The cows were machine stripped. The intact milking unit was taken immediately into the milk house for sampling and weighing the milk. The milk then was carefully transferred to identified sterile covered 10-gallon cans. The milk was not strained. The machine



was partially dismantled and all parts which came in contact with milk were rinsed twice in warm tap water, followed by an immersion rinse in a solution containing 200 p.p.m. available chlorine. The reassembled unit then was returned for immediate use on other animals. Terminating each milking period, the machines were water rinsed, completely disassembled, washed and scrubbed, rinsed in hot water, and stored on racks for later milking periods.

For hand milking, open-top oval dairy pails were sterilized, as were the milking machine pails. The cows' udders were wiped in the same manner as when machine milked. The six milkers immersed their hands in a solution containing 200 p.p.m. chlorine prior to each milking, after which they touched nothing except the cows' teats until each milking assignment was completed. The milking stools were handled for the milkers by assistants. To obtain balanced results, the milkers alternated at random between clipped and non-clipped cows. The milk obtained by hand milking of each cow was immediately transferred to the milk house for sampling.

*Milk sampling procedures.* The following procedures were used for bacteriological analysis of the milk: (a) A sample from each pail of milk from each cow was obtained immediately after it was conveyed to the milk house. (b) Separate composite samples of the milk from the clipped and non-clipped cows, respectively, were taken immediately from each 10-gallon can filled during the milkings. (c) Separate composite samples and off-the-bottom sediment tests of the milk from the clipped and non-clipped cows were taken from each can at the time the milk was delivered to the dairy plant.

The samples of milk, transferred by means of a sterile glass tube thief to sterile screw cap bottles, immediately were placed in ice water and so kept until the bacteriological tests were undertaken, regularly within 60 minutes. The filled milk cans were stored in a water immersion refrigerator at 33 and 34° F. The milk in cans was held until approximately 7:00 a.m., when it was trucked to the dairy plant, a distance of approximately 0.25 mile (milkings were begun at 3:30 a.m. and p.m.).

*Methods of testing.* Standard bacteria plate count estimates of the milk were made as described in Standard Methods for the Examination of Dairy Products, 8th Edition (1). The raw milk samples were plated in duplicate at dilutions of one in ten. Samples pasteurized in the laboratory were plated without dilution. The milk was pasteurized at 143° F. in a thermo controlled bath for 30 minutes, using 5-ml. portions and a blank open tube for thermometer observation. All counts are reported as bacteria per ml.

The presence of extraneous material in the milk was determined by use of a Langsenkamp-Wheeler off-the-bottom sediment tester which withdrew a 1-pint sample from each 10-gallon can of composited milk.

## RESULTS

In table 1 are presented the bacteria counts of the milk (weighted arithmetic averages) from those cows which were milked throughout the first and the second periods. In the first or control period, all udders were unclipped; in the second period, udders of alternate cows were clipped. The average count per ml. of the milk from those cows not clipped was determined as 1,308 in the control period and 1,869 per ml. in the second

TABLE 1

*Weighted arithmetic average count per ml. of samples of milk taken from individual milkings obtained by machine during preliminary period and after clipping part of cows*

Treatment after preliminary period	No. of cows	Prior to clipping any cows		After clipping part of the cows	
		No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
Unclipped .....	10	19	1308	84	1869
Clipped .....	13	22	1629	92	1397

period. The count per ml. of the milk from cows subsequently clipped decreased from the control period average of 1,629 to 1,397. Since, during the second period, the average count of milk from the cows remaining unclipped increased, while that of the milk from the cows clipped decreased, the apparent over-all difference in numbers of organisms in the milk appears to indicate beneficial effects of clipping.

In table 2 are presented the weighted arithmetic averages of the counts per ml. of milk from cows during the second period, when milked by machine, and during the third period, when milked by hand. The results are presented on the basis of both morning and evening milkings. The number of cows involved in this analysis varied due to drying-off and

TABLE 2

*Weighted arithmetic average count per ml. of samples of milk taken from individual milkings*

Time of milking	Clipped			Unclipped		
	No. of cows	No. of samples	Weighted arithmetic av. count per ml.	No. of cows	No. of samples	Weighted arithmetic av. count per ml.
<i>Machine milked</i>						
Evening .....	13	92	1548	23	115	1805
Morning .....	13	93	1375	16	101	1317
<i>Hand milked</i>						
Evening .....	14	83	877	16	83	1484
Morning .....	14	102	830	16	104	1143

TABLE 3

*Weighted arithmetic count per ml. of composite samples of milk from clipped and unclipped groups of cows*

Time of milking	Clipped		Unclipped	
	No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
<i>Machine milked</i>				
Evening .....	8	1590	8	2381
Morning .....	8	1254	8	1245
<i>Hand milked</i>				
Evening .....	6	566	7	1250
Morning .....	8	771	8	1000

freshening. In addition to these natural causes, some samples were omitted because of explainable contamination, such as dropping of teat cups into the bedding, cows kicking into milk pails during hand milking, and sudden evidence of mastitis. The results show that the average count per ml. of the milk obtained by machine milking from clipped cows differed but slightly (4.4 per cent more in morning and 2.4 per cent less in evening) from that similarly obtained from unclipped cows. On the other hand, the results show that the average counts of the milk obtained by hand milking from clipped cows were less (40.9 per cent for morning and 28.0 per cent for evening) than those similarly obtained from unclipped cows.

In table 3 are presented the average counts per ml. of the composite samples of milk obtained from filled 10-gallon cans. The average count per ml. of this milk from the clipped cows was definitely less than that from the non-clipped cows. The over-all difference approximated 30 per cent. A similar relationship in bacteria numbers of the evening's milk refrigerated for approximately 14 hours before being sampled also was

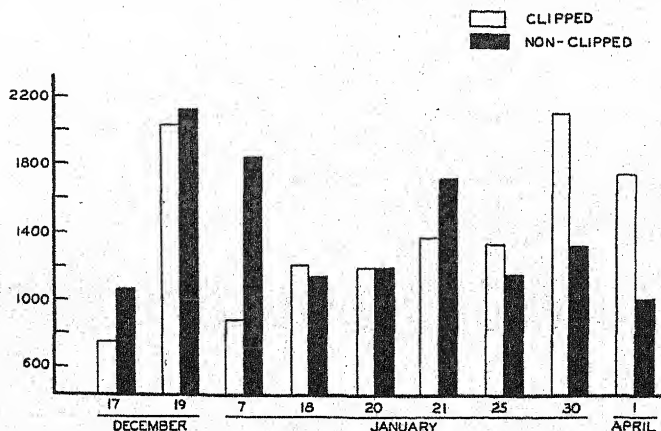


FIG. 2. Average bacteria count of individual milkings of cows milked by machine in morning.



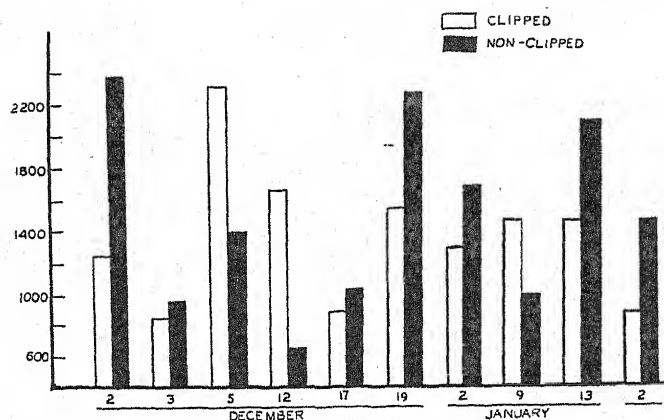


FIG. 3. Average bacteria count of individual milkings of cows milked by machine in evening.

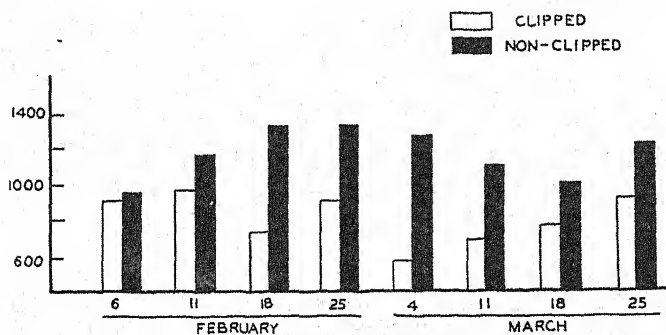


FIG. 4. Average bacteria count of individual milkings of cows milked by hand in morning.

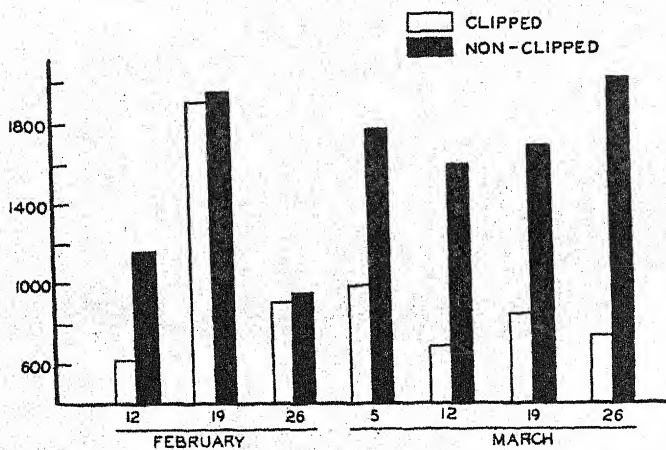


FIG. 5. Average bacteria count of individual milkings of cows milked by hand in evening.

observed. The counts of the composite samples subsequently pasteurized ranged from 3 to 13 per ml. and were so low that no significance could be attached to the figures.

The average counts per ml. of the milkings from the clipped and non-clipped cows, during the morning and evening milking, for each milking period included in the study, are presented in figures 2, 3, 4 and 5. An analysis of variance of the data presented in the graphs shows there is a significant difference in the count per ml. of the milks obtained by hand milking between the clipped and non-clipped cows. The difference in the counts per ml. of the milks obtained by machine milking from clipped and non-clipped cows was not statistically significant.

The tests for extraneous material present in the milks were conducted at the time the milks were delivered to the platform of the plant. No

TABLE 4  
*Frequency of the grades (Wisconsin Standards) of the tests  
for extraneous material in milks*

	Grades			
	1	2	3	4
<i>Machine milking</i>				
Clipped cows .....	1	16	7	1
Unclipped cows .....	.....	14	10	0
<i>Hand milking</i>				
Clipped cows .....	.....	.....	8	20
Unclipped cows .....	.....	.....	5	18

difference in milks from clipped or unclipped cows could be determined. However, the amount of extraneous material in the milk obtained by hand milking was much greater than in that obtained by machine. The summary of the tests is tabulated in table 4.

The udders usually were clean prior to washing except for loose dirt or shavings clinging to the hair. The time spent in washing the udders prior to milking did not differ appreciably between the clipped and unclipped cows. This was used as the routine stimulus for let-down of milk, and the time spent was more than adequate to cleanse the teats and udder of all visible dirt.

#### DISCUSSION AND SUMMARY

The effect of the clipping of cows upon the quality of milk was determined by colony plate count and tests for presence of extraneous material. The clipping tended to lower the average bacteria counts per ml. of the milk, whether the milking was done by machine or by hand. The average counts per ml. of the milk obtained by machine from clipped and unclipped cows were 3,042 and 3,458, and by hand 1,643 and 2,996, respectively. The advantages of clipping were statistically significant for the milks obtained by hand milking.

The average counts per ml. of the milk obtained by machine were greater than those of the milk obtained by hand. This might be due to the ends of the teats being bathed to some extent by milk during machine milking, resulting in rinsing of organisms into the milk. When the milking is performed by hand, the bathing action does not occur. Although the average bacteria count per ml. of the milk obtained by machine was greater than when obtained by hand, the amount of extraneous material present was observed to be greater in the milk obtained by hand. The clipping of cows caused no measurable difference in the amount of extraneous material in milk when obtained by either machine or hand milking.

#### REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th Ed. American Public Health Assoc., New York, N. Y. 1941.
- (2) BRYAN, C. S. Dairy Bacteriology and Public Health Handbook. Burgess Publishing Company, Minneapolis, Minnesota. 1945.
- (3) HARDING, H. A., RUEHLE, G. L., WILSON, J. D., AND SMITH, G. A. N. Y. (Geneva) Agr. Expt. Sta. Bull. 365. 1913.



## STORAGE AND TREATMENT OF MILKING MACHINE INFLATIONS UNDER FARM CONDITIONS<sup>1,2</sup>

J. M. JENSEN AND A. L. BORTREE

*Michigan State College, East Lansing*

Difficulty in producing high quality milk frequently has been attributed to using milking machines that were not in the proper state of sanitation. Many procedures have been advocated as a means of sanitizing the rubber inflations and tubing. Most of these procedures were discussed in a previous article (3) in which the authors presented results of a laboratory study of rubber inflations for milking machines. The study reported: (a) the extent of fat absorption, (b) the extent of storage solution absorption, (c) the deteriorating effect of inorganic chlorine, and (d) the advantage of boiling rubber parts in lye solution at intervals as a means of saponifying entrained fat, thus improving their sanitary condition.

Mallman *et al.* (4) recently reported a study in which producers used a variety of sanitizers. This study indicated that cationic germicides were more effective as sanitizing agents than lye and chlorine, as measured by lower total and thermoturic counts of milk.

Dahlberg (2) also recently reported results of milking machine sanitizing studies and found high bacterial counts with dry-stored inflations and low counts and clean tubes with lye solution on rack storage. He reported that dry storage following washing and rinsing with cationic germicides was not satisfactory.

Investigation seemed desirable to determine certain storage and treatment practices under practical farm conditions. To this end farm studies were made over an extended period on the treatment and storage of rubber inflations and tubing of milking machines, studying the physical and bacteriological cleanliness of the rubber and the bacterial population of milk produced through their use.

### EXPERIMENTAL PROCEDURE

*Preliminary observation of producer methods.* Producers of milk from one dairy plant were used for this study over an 18-month period. The early portion of the study consisted of making inspection of milking machines for cleanliness and method employed for storage between use, while weekly bacterial counts were made on milk from each producer.

Received for publication December 10, 1947.

<sup>1</sup> Journal Article no. 919 (n.s.), Michigan Agricultural Experiment Station.

<sup>2</sup> This study was made possible through a grant from Swift and Company, Chicago, Illinois, for research in quality milk and cream.

During this period certain storage practices were found to give satisfactory results and others were observed to have some objectionable features that deemed them undesirable for rubber storage. The producers having generally satisfactory results were those using lye and cationic germicide solutions, and dry storage following scalding with very hot water. Acid solution had no appreciable germicidal property and inorganic chlorine caused gross deterioration of rubber. This portion of the study was continued by making bacterial counts of sterile water rinses of the inspected rubber parts and bacterial counts of the milk produced through their use.

Based on these observations, further study was made comparing dry, lye and cationic solution storage under conditions wherein the washing procedure employed before their use would be the same. Producers operating two milking machines each were chosen for this study. Four different storage treatments were compared: (a) dry storage following the regular washing procedure, (b) dry storage following washing and rinsing, after which a subsequent rinse with 1 gallon of 200 p.p.m. cationic germicide solution was used, (c) washing followed by solution storage, 200 p.p.m. cationic germicide, and (d) solution storage with 0.5 per cent lye solution. All milking machines were washed using a washing powder consisting of anionic synthetic detergent and near-neutral polyphosphate. Long tube milkers generally were washed by a flush washing procedure, while short tube milkers generally were washed disassembled.

Each producer having two milker units was asked to follow different designated storage procedures on each unit as a means of comparing the sanitary condition of the inflations and tubing affected thereby, thus eliminating the factor of washing effectiveness, which was assumed to be the same for both units. Examinations and bacterial counts were made over a period of 5 to 10 weeks, the greatest number involving ten weekly examinations.

Bacterial counts of inflations were made from water rinses, using 500 ml. of sterilized water, chilled by icing to ice water temperature. This water was emptied into the cups and tubing of long tube milkers while supporting these parts in an upright position. After filling, the water was returned to the water jar by discharging through the rubber tubing. Short tube milker inflations were filled after pinching shut the tubing near the outlet. Four inflations from each short tub milker unit were rinsed similarly. The samples again were placed in iced water and within 4 hours were plated for total count, using standard methods (1). Thermoduric counts of rinse water were made by plating the water after laboratory pasteurization at 143° F. for 30 minutes.

Weigh-can samples of each producer's milk supply were taken the day after examining inflations. These were plated for total and thermoduric count. Examination for physical cleanliness of inflations and tubing was

made by scraping the inner lining of inflations with a spatula and rodding the tubing. Evidence of soiling was noted.

## RESULTS

*Use of dry storage with and without cationic germicide rinse.* Bacterial counts resulting from dry storage as obtained from three producers are shown in table 1. A survey of bacterial counts of inflations shows no important improvement in sanitation when 200 p.p.m. cationic germicide solutions were used for rinsing following washing. This was true when high bacterial counts were obtained with producers 3L and 17L, as well as when low counts were secured by producer 36L, whose low counts were attributed to rinsing the washed utensils with a liberal quantity of very

TABLE 1  
*Influence of cationic rinse on bacterial population of rubber inflations*

Producer	Method of storage <sup>a</sup>	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoturic	Total	Thermoturic
36L <sup>b</sup>	A	10	5,000	1,300	25,000	600
	B	10	3,000	1,500	100,000	600
	C	10	4,000	1,200		
3L	A	11	550,000	4,600	40,000	1,300
	B	9	220,000	2,100	46,000	500
	C	9	650,000	1,600		
17L	B	5	2,800,000	5,600		
	C	5	1,400,000	5,000	160,000	12,000

<sup>a</sup> A = dry, preliminary trials; B = dry; C = dry, cationic germicide rinse.

<sup>b</sup> L = long tube milker.

hot water. Total and thermoturic counts of inflation rinses from producers 3L and 17L were excessively high, and the cationic rinse gave insufficient germicidal property by this method of application.

Total and thermoturic counts of milk produced with the inflations also are shown in the table. No attempt was made to relate these counts to the particular sanitizing treatment applied to inflation assemblies, since it was not practical to segregate milk produced by each milker unit. Normally low thermoturic counts were obtained from producers 36L and 3L, while those of 17L were high, as were also the total counts of inflations from this producer. Averages obscure the conditions that were involved in this producer's counts. These conditions are presented in detail in table 5.

*Dry storage versus storage in cationic solution.* Bacterial counts of rubber inflations and tubing secured when comparing dry storage following washing with solution storage using 200 p.p.m. cationic germicide solution are shown in table 2.



Three producers' machines were observed. The most pronounced difference in results was secured with producer 1S, for whom cationic solution storage results were very satisfactory, with an average count of 11,000 for the nine trials. The dry storage inflations, while similarly washed, were excessively high in count, averaging 3,800,000 for the nine trials. The extreme difference secured with this producer is accounted for by the observation that washing and rinsing were done with medium warm water and no attempt was made to sanitize the dry storage inflations.

Producers 2S and 26L had low bacterial counts on both dry and cationic solution trials, counts that were markedly lower than in the preceding preliminary trials. Both producers were habitually careful about cleaning their milkers and were discovered to be somewhat reluctant to

TABLE 2

*Influence of cationic solution storage on bacterial population of rubber inflations*

Producer	Method of storage <sup>a</sup>	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoturic	Total	Thermoturic
1S <sup>b</sup>	A	9	170,000	3,100	39,000	700
	B	9	3,800,000	4,700	250,000	5,200
	C	9	11,000	1,700		
2S	A	7	30,000	3,000	35,000	800
	B	10	2,500	1,000	11,000	500
	C	10	3,500	2,300		
26L	A	7	58,000	1,900	230,000	1,300
	B	6	1,300	700	830,000	900
	C	6	500	500		

<sup>a</sup> A = dry, preliminary trials; B = dry; C = cationic solution.

<sup>b</sup> S = short tube milker; L = long tube milker.

use sanitizing solution storage. They therefore were concerned about having the dry storage inflations as bacteria-free as those stored with cationic solution and used very hot water for sanitizing following washing.

All of the machines in table 2 were washed disassembled. Machines and inflations of producers 2S and 26L always were found in an excellent state of cleanliness, while those of 1S at times were criticized for being slightly slimy in the upper portion of the inflations.

The conditions of the milking machine tubing and inflations were not necessarily reflected in the total bacterial counts of the milk. High bacterial counts were obtained in milk samples from producer 26L, in spite of his use of clean and well sanitized inflations and tubing. The high counts were attributed to delayed and inadequate cooling caused by the milk house being located near the farm residence across a highway from the barn. Also, this producer had more milk than could be accommodated in his cooling tank. However, there was considerable increase in thermoturic

count on the milk in samples from producer 1S when the milker inflation total counts were high.

Trials comparing solution storage using 0.5 per cent lye and 200 p.p.m. cationic germicide are shown by bacterial counts listed in table 3. Most apparent is a reduction of bacterial count when solution storage was used, in contrast to the situation when dry storage was employed by the various producers during the preliminary trials.

When long tube milkers requiring solution racks were used for storage of inflations and tubing, as shown by producers 5L, 9L, 15L, 23L, and 59L

TABLE 3

*Influence of cationic solution and lye solution storage on bacterial population of rubber inflations*

Producer	Method of storage <sup>a</sup>	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoturic	Total	Thermoturic
5L	A	11	7,800	1,400	47,000	1,200
	B	9	2,000	800	48,000	6,200
	C	9	2,000	1,000		
9L	B	6	5,900	800	31,000	600
	C	6	6,600	600		
15L	A	11	390,000	6,000	9,600	1,000
	B	9	1,400	1,500	10,000	600
	C	9	1,400	600		
23L	A	11	140,000	3,500	100,000	
	B	8	1,100	800	83,000	900
	C	8	1,300	500		
59L	A	8	170,000	13,000		
	B	9	7,600	2,800	28,000	400
	C	9	1,100	600		
61S	D <sup>b</sup>	11	13,000	4,000	44,000	4,700
	B	9	24,000	1,900	10,000	600
	C	9	1,300	800		

<sup>a</sup> A = dry, preliminary trials; B = lye solution; C = cationic solution.

<sup>b</sup> D = lye solution, preliminary trials.

lye and cationic germicide solutions were equally effective as sanitizing agents, as indicated by total bacterial counts of rinses. A consistent, though small, decrease in thermoturic bacterial counts of rinse samples was secured in favor of cationic germicide solution storage over lye solution storage. Likewise, there was a general reduction in milk thermoturic counts when solution storage was used compared with dry storage. One exception to this is observed in producer 5L. Although the counts on the inflations and tubing were low, this producer was not successful in maintaining general physical cleanliness of the rubber parts of his machines. Usually spatula scrapings from the inflations gave heavy milk sludge deposits. This condition was found to be caused by dipping milk-coated

teat cups in warm chlorine solution of 200 p.p.m. strength before milking the next cow, without previously rinsing off the milk in cold water. Such a treatment caused a slimy film that was not removed readily during washing.

When inflations were stored in solution jars, as was required of the short tube inflations of producer 618, cationic solution storage was more effective than lye as a sanitizing agent. The inflations stored in the cationic solution yielded an average rinse count of 1,300 in contrast to 24,000 for lye storage.

Higher rinse counts were secured using lye in jar storage that were obtained with lye used in solution racks. This was considered to be due to repeated use of the same lye solution for a 7-day period in jar storage, whereas a fresh lye solution was applied between each milking when rack storage was used.

*Physical cleanliness and bacterial cleanliness.* A tabulation of the

TABLE 4  
*The relationship between bacterial count and physical cleanliness of milker inflations*

Bacterial count of milker inflations	Appearance of milker inflations			
	Clean		Not clean	
	No.	%	No.	%
<10,000	242	51.0	34	24
10,000—100,000	97	20.4	25	18
100,000—1,000,000	88	18.3	33	23
>1,000,000	49	10.3	50	35

milking machines examined over the entire course of the study was made to determine to what extent cleanliness by physical examination was verified by the bacterial counts obtained. This included 617 examinations. Of this number, 475 were noted as being clean and 142 as not clean. The bacterial counts of both groups are shown in table 4. In accordance with the data presented, it would appear that milking machines can be judged for bacteriological cleanliness by physical examination with only a fair degree of success, for in 51 per cent of the milkers rated clean, the bacterial counts were less than 10,000, which probably could be considered a "fair" count for inflations stored dry. However, 29 per cent of the milkers that appeared clean were highly contaminated.

There was less relationship between appearance of milker inflations in the "not clean" group. Here 24 per cent had counts of less than 10,000. These figures likely were not representative of average conditions, since the number of samples was relatively small and among them were inflations that frequently were found to retain an oily wax-like deposit as the result of storage in cationic germicide and the inflations of producer 5L



that were doused with warm chlorine solution without first rinsing off adhering milk with clear water.

*Producer reaction.* Some objections to cationic solutions were expressed by producers. One expressed dislike for cationic detergents because they made the rubber feel "dead" and because they caused the rubber to become coated with a slippery film. Occasionally this would cause the milk pail gaskets to be sucked into the milker pails. It was discovered that this condition occurred mainly when the milk films were not completely washed off the rubber before placing the parts in the solution jar. Lye solution was preferred by this operator because it served better as a detergent.

TABLE 5

*The relationship between sanitization treatment and bacterial condition of a single set of milking machine inflations (producer 17L)*

Date	Bacterial count/ml.			
	Inflations		Milk	
	Total	Thermoturic	Total	Thermoturic
2-5 <sup>a</sup>	3,000	1,000	10,000	2,400
2-19 <sup>a</sup>	10,000	500	16,000	500
3-27 <sup>b</sup>	310,000	5,000	30,000	400
	510,000 <sup>c</sup>	6,000		
4-3 <sup>b</sup>	52,000	5,000		300
	200,000 <sup>c</sup>	3,000	7,000	
4-10 <sup>b</sup>	1,000,000	2,000		4,000
	1,300,000 <sup>c</sup>	2,500	88,000	
4-16 <sup>b</sup>	10,000,000	2,500		600,000
	75,000,000 <sup>c</sup>	2,500	930,000	
4-24 <sup>b</sup>	30,000,000	25,000		900,000
	21,000,000 <sup>c</sup>	50,000	6,000,000	
5-1 <sup>a</sup>	3,500 <sup>d</sup>	4,500		400
	10,000	4,000	22,000	

<sup>a</sup> Hot water used.

<sup>b</sup> Cold water used.

<sup>c</sup> Rinsed with 200 p.p.m. cationic solution after washing.

<sup>d</sup> Stored on solution rack with 200 p.p.m. cationic solution.

Some objection also was expressed with respect to cationic solution forming an oily and somewhat wax-like film when used for sanitizing milker pails. This condition also was found inside inflations. At no time during the course of this study was milkstone a problem. Only during the initial portion of the study was it noticed. The anionic detergent combined with near-neutral polyphosphate was effective in its removal as well as in its prevention. A soft gel-like slime occasionally was found when examining inflations for cleanliness by spatula scraping. This usually was found when chlorine solutions were used for sanitizing without properly removing all milk film by rinsing in water.

Most difficulty with bacterial contamination occurred where washing with detergent and cold or only moderately warm water was used, after which the inflations were stored dry. An illustration of the result of such washing was well demonstrated by producer 17L, who had been producing milk for several months with a good record of low bacterial counts. His hot water heater was sent away for repair for several weeks during the course of the study. Results as shown in table 5 reveal a progressive degree of contamination that first was made evident by increase in the rinse count of inflations. Milk samples remained normal during the first 2 weeks of cold water washing and then greatly increased in total and thermoduric counts. Immediate reduction in counts followed the return to hot water rinsing after washing. Similar patterns were discernible with other producers that washed their machines but did not follow up with effective germicidal treatment. This would support a concept that inflations are not a formidable source of bacteria until contamination has penetrated the rubber pores.

#### DISCUSSION

The results of storing rubber milker inflations under practical farm conditions indicate that lye and cationic germicide solution storage provide an assurance of better sanitation than does dry storage. Germicidal properties of 0.5 per cent lye and 200 p.p.m. cationic germicidal solutions appear to be of practically equal value where fresh solutions of lye are applied, such as is made possible with solution rack storage. When jar or bath storage was used, cationic solutions provided better sanitizing than did lye. This would indicate that the cationic solutions are more durable as germicides than lye and that when lye jar storage is used for rubber parts, fresh solutions should be prepared more frequently than once each week, as was used in the study.

The cationic solution applied as a rinse following washing was not satisfactory as a means of sanitizing the rubber parts. This was indicated by high bacterial counts in the inflations as well as in milk produced with the inflations. Apparently more intimate exposure to the cationic germicide was required than was possible by drawing 1 gallon of solution through the cups and tubing.

The authors previously reported (3) that rubber inflations were capable of absorbing storage solutions to an appreciable degree. Butterfat and unquestionably bacterial contamination likewise have been found to be absorbed by rubber. With these absorbing qualities definitely shown, it would seem logical to conclude that the problem of sanitizing rubber is not just a matter of treating the surface but of penetrating the pores either by prolonged contact with penetrable germicidal solution or with heat. Hence, the lack of effective germicidal treatment by rinsing and

retaining only a surface film could be expected. It likewise would follow that a combined washing-sanitizing compound, such as frequently has been sought, would not prove effective unless followed by solution storage.

The observations indicate that rinse counts do not represent the entire bacterial contamination but only a portion of that present on the surface of the rubber. Low rinse counts therefore should not be accepted with complete assurance that inflations are sanitized satisfactorily unless the milk produced with them yields low total and thermoduric counts.

In this study, milker pails were not examined for bacterial contamination but only for appearance of cleanliness. General observation led to the belief that the greatest contamination came from milker inflations and tubing.

#### CONCLUSIONS

Farm application of milker inflation storage employing 0.5 per cent lye solution, 200 p.p.m. cationic germicide and dry storage was observed through means of farm inspection, sterile rinse counts of inflations and bacterial counts of milk. Comparison of storage treatment was made using two different procedures on each of two designated milkers on each farm.

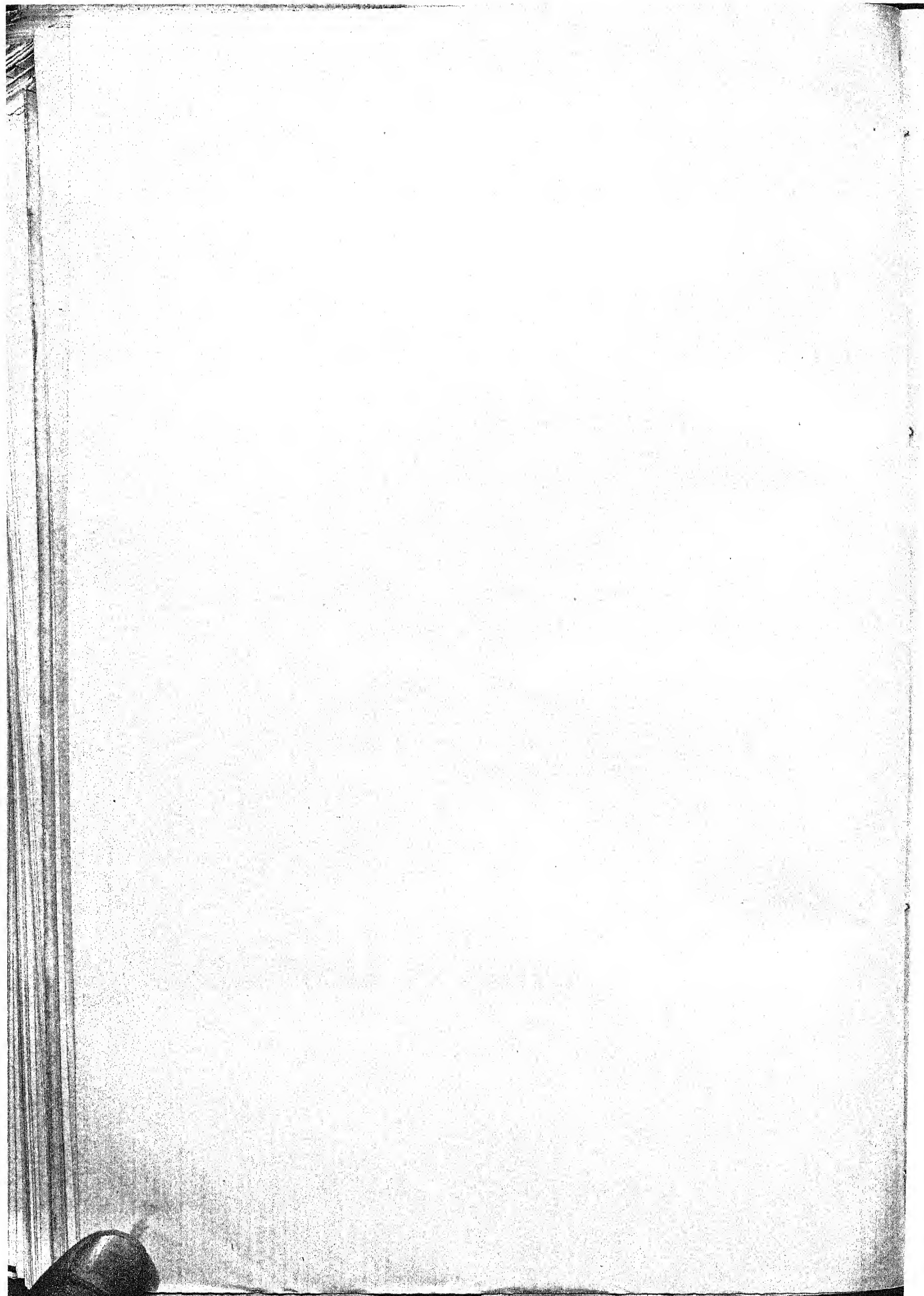
The lye and cationic solutions appeared to have equal germicidal value as measured by total rinse counts when solution rack storage was used. The cationic germicide solution caused greater reduction in thermoduric count of rinse water samples than did lye. Lye solution had less germicidal effectiveness than the cationic germicide when immersion storage was used.

Dry storage was least satisfactory in maintaining uniformly low counts of inflations, and high thermoduric counts were associated with cold water washing followed by dry storage. Dry storage after washing and "sanitizing" with 1 gallon of 200 p.p.m. cationic solution was not satisfactory. Some objection to physical properties of the cationic germicide was registered.

#### REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Milk. 8th Ed. Amer. Pub. Health Assoc., New York. Pp. 14-39. 1941.
- (2) DAHLBERG, A. C. The Sanitizing of Milking Machines. Abstract, Dairy Inds. Conference, Cornell Univ. 1947.
- (3) JENSEN, J. M., AND BORTREE, A. L. Storage and Treatment of Milking Machine Inflations. *J. Dairy Sci.*, 29: 849-859. 1946.
- (4) MALLMAN, W. L., KIVELA, E., BORTREE, A. L., CHURCHILL, E., AND BEGEMAN, L. H. The Influence of the Method of Sanitizing Milking Machines on the Bacterial Content of Milk. *Ann. Rept. N. Y. State Assoc. Milk Sanitarians*, 20: 177-194. 1946.





## SWEET POTATO MEAL VERSUS GROUND CORN IN THE RATION OF DAIRY COWS<sup>1</sup>

JENNINGS B. FRYE, JR., JOHN H. THOMASON, AND HERBERT B. HENDERSON

*Dairy Department, University of Georgia, Athens*

Raw sweet potatoes have been used as a feed for domestic animals for some time. However, it has been only during the past decade that sweet potato meal has gained recognition as a possible substitute for corn in the ration of livestock. Several southern investigators (6, 7, 8, 9, 10, 11, 12, 13) have found dried sweet potatoes to be worth approximately 90 to 95 per cent as much as corn for fattening beef cattle. Hogs and mules preferred rations in which the sweet potato meal did not replace more than 50 per cent of the corn in the ration. Massey (14) at the Georgia Experiment Station conducted a series of experiments in which he studied the comparative value of sweet potato meal and ground corn as a feed for ewes. The 300 ewes that were fed these rations during a period of 3 years favored the sweet potato meal from a palatability standpoint and gained as much during the gestation period when fed the sweet potato meal ration as when fed the corn meal ration. The ewes that received the sweet potato meal ration produced more milk after the lambs were born and thereby gave the lambs a faster growing start.

Louisiana workers (15) report that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal and are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck, for milk production. Good quality dehydrated sweet potatoes contained from 76 to 81 per cent T.D.N. and a poor quality product contained 71 per cent T.D.N. on the dry basis. Vitamin A analyses of the butterfat from milk produced by cows fed sweet potatoes were 19 per cent higher than those of butterfat produced by cows fed ground corn. The basal feeds were common lespedeza hay, alyce clover hay, or kobe lespedeza hay and cottonseed meal. Briggs *et al.* (2) of the Oklahoma Station recently reported that on the dry matter basis the average T.D.N. value of dried sweet potatoes for steers was 86.05 as compared to 85.80 for the corn. The basal feeds were alfalfa hay or prairie hay and cottonseed meal. Copeland (5) of Texas reported that one could expect 3.08 per cent more milk as a result of feeding corn than when feeding sweet potato meal. It also was noted that butter produced from cows on the sweet potato meal contained 37.98 I.U. of vitamin A per g. as compared with 31.11 I.U. from butter produced by cows fed yellow corn.

Received for publication December 13, 1947.

<sup>1</sup> This paper is part of a thesis presented by John H. Thomason to the Graduate School of the University of Georgia in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

Massey (14), in a study with 48 cows, reports that the cows which received the sweet potato meal ration produced 9.3 per cent more milk than did those fed the corn ration.

#### EXPERIMENTAL PROCEDURE

In the spring of 1946, eight Jersey cows of the University of Georgia dairy herd were paired into two groups (Groups A and B) and fed comparative rations of sweet potato meal and ground snapped white corn. The constituents of the concentrate mixtures used in the experiment are shown in table 1. The mixtures were the same except that an equal amount of sweet potato meal was substituted for the corn in mixture 2 and equal parts of corn and sweet potato meal were used in mixture 3. The cows also were fed mixed lespedeza and grass hay *ad libitum*. The ani-

TABLE 1  
*Constituents of grain mixtures used in experiment*

Constituents	Mixture 1	Mixture 2	Mixture 3
	(lb.)	(lb.)	(lb.)
Ground snapped white corn .....	200		100
Sweet potato meal .....		200	100
Oats .....	100	100	100
Wheat bran .....	100	100	100
Cottonseed meal .....	135	135	135
Bonemeal .....	9	9	9
Salt .....	4.5	4.5	4.5

mals were quartered in a dry lot except when being milked by machine twice daily.

The double reversal method of experimentation (1, 4, 18) was employed in this work. Both Groups A and B were fed mixture 3, which contained equal parts of corn and sweet potato meal, during a preliminary period of 14 days. Then Groups A and B were fed mixtures 1 and 2, respectively, during the first experimental period. The groups were changed alternately from one ration to the other during the second, third, and fourth experimental periods, each of which extended for 28 days.

When the experiment was about one-third over, cows no. 7 and 8 had to be withdrawn from the experiment due to the incidence of mastitis. In view of the fact that these two cows were paired together before the experiment started, their removal affected the experiment only insofar as the number of cows in each group was reduced by one.

Analysis of variance (4) was used in the statistical treatment of the liveweight and milk and butterfat production data according to two different methods. Method 1 tripled the differences between the response of the cows on the two feeds during the second and third experimental periods ( $-a + 3b - 3c + d$ ) and gave equal weight to the differences during the first and



fourth periods. Method 2 (3) consisted of giving equal weight ( $a-b + c-d$ ) to the differences incurred during each experimental period.

#### RESULTS

*Chemical analysis of feeds.* Sufficient quantities of both rations were mixed bimonthly so as to keep a fresh supply of feed on hand at all times. A representative sample from each batch of feed was analyzed for dry matter, ether extract, crude protein, nitrogen-free extract, crude fiber, ash and moisture. The data shown in table 2 reveal that the corn ration contained 0.70 per cent more moisture, 1.6 per cent more fiber, 0.50 per cent more protein, 0.30 per cent more fat, 1.0 per cent less ash and 2.20 per cent less nitrogen-free extract than did the sweet potato meal ration. Very little difference in the chemical composition of the two mixtures was apparent.

The work of Briggs *et al.* (2) and Rusoff *et al.* (15) indicates that the

TABLE 2  
*Average chemical analyses of experimental rations<sup>a</sup>*

Mixture	Constituents					
	Moisture	Fiber	Protein	Ash	Fat	Nitrogen-free extract
1	9.8	8.8	16.9	5.2	4.3	55.0
2	9.1	7.2	16.4	6.2	4.0	57.2

<sup>a</sup> Chemical analyses were made by personnel of the State Chemists Department.

apparent digestion of coefficients of the protein, fat and fiber of dehydrated sweet potato meal may be rather low. The Louisiana workers (15) point out that since the N.F.E. made up over 84 per cent of the dry matter in the dehydrated sweet potato, the apparent lack of digestibility of the other constituents had but little effect on the T.D.N. content and that the low content of protein precludes this product from being an important source of this nutrient. It would appear from these reports (2, 15) that the sweet potato meal ration (mixture 2) fed in this experiment may have been a little lower in digestible protein than the corn ration (mixture 1).

*Palatability.* All of the cows relished the sweet potato meal (Porto Rico variety) ration from the start of the experiment; when the cows were changed from one ration to the other, they did not eat the corn ration as readily as they did the sweet potato meal ration. This is in agreement with the results obtained by Massey (14) of the Georgia Experiment Station and Seath (16) and Seath *et al.* (17). The Porto Rico variety of sweet potato was fed in these studies. The Louisiana workers (17) observed in another study that, when the high-starch sweet potato variety, L-45, was compared with ground yellow corn meal in the ration,

from 1 to 4 days were required for all of the cows to become accustomed to the change in the rations. Then they ate the ration containing sweet potato meal as readily as they did the one containing corn meal. In regard to the varying degrees of palatability of sweet potato meal as reported, one should remember that this product is a relatively new livestock feed, the quality of which has not yet been standardized. The proportion of sweet potato meal to other constituents and the number of various ingredients used in the ration probably would have a definite relationship to the palatability of the ration containing the sweet potato meal. From the standpoint of color and palatability, the product used in this experiment was excellent.

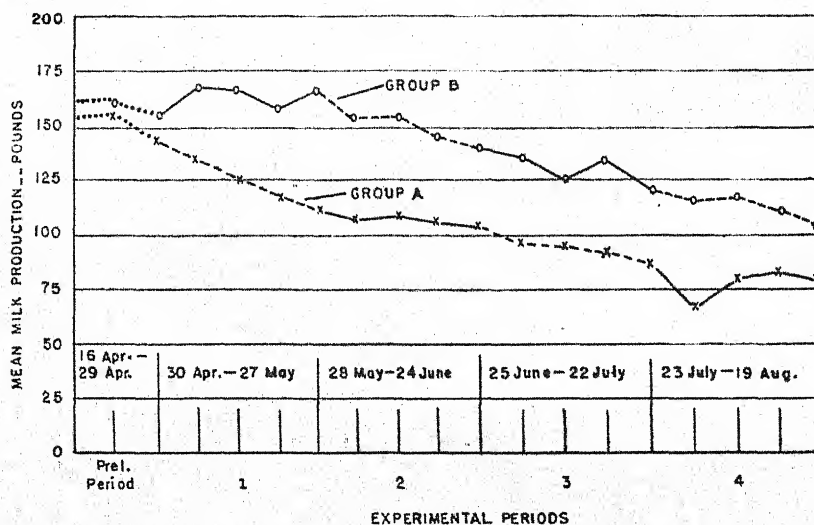


FIG. 1. Curves showing mean milk yield of cows on experiment. (Group A = X; Group B = O. Mixture 1 = -----; Mixture 2 = —————; Mixture 3 = .....)

The cows fed the sweet potato meal appeared to have a sleeker hair coat than did those fed the corn ration. There was no indication of any unusual looseness in the feces of the animals as the result of feeding the sweet potato meal; the feces of these cows were not as loose as those from the regular herd cows which were on green pasture. Massey (14) likewise found no digestive disturbances among the cows fed sweet potato meal, although it seemed to have a more laxative effect than did the corn meal.

*Liveweight.* The liveweight average per cow when fed the corn ration was 798 lb. as compared to 801 lb. when fed the sweet potato meal ration. Statistical treatment of the data according to the methods described previously gave *F*-tests which indicate that there was no significant difference between the effect of the two feeds on the liveweights of the cows.

*Milk and butterfat yields.* A comparative study of the effect of corn and sweet potato meal on milk and butterfat production was made during each of the experimental periods. The milk production data (Fig. 1) reveal that although the level of production of Group B was higher than that of Group A, the trend of the lactation curves of the two groups of cows was very much the same throughout the experiment. The level of production of the two groups was about the same at the start of the study. The sharp drop in the curve of Group A during the period July 23 through July 29, inclusive, was attributed to a case of foot rot, which one of the cows had during that period. Group A was being fed sweet potato meal ration during this period.

From the standpoint of total production, when the cows were fed the corn ration (mixture 1) they produced 5,752.1 lb. of milk as compared to 5,741.2 lb. when fed the sweet potato meal ration (mixture 2). The butterfat yields of the cows when fed each of the experimental rations were calculated from a bimonthly butterfat test and the actual milk production. When fed the corn ration, the cows produced a total of 267.58 lb. of butterfat as compared to 268.72 lb. when fed the sweet potato meal ration. The *F*-tests indicate that there was no significant difference between the effects of the two feeds on the amount of milk and butterfat produced.

#### SUMMARY

A comparative study of the effect of ground snapped white corn and sweet potato meal on the liveweight and milk and butterfat production of dairy cows was conducted during the spring and summer of 1946. Analysis of variance revealed no significant differences in the milk and butterfat production or in the liveweights of cows when the sweet potato meal or corn constituted 36 per cent of the concentrate mixture. The sweet potato meal was as palatable as the ground corn when each of these concentrates constituted 36 per cent of the concentrate mixture. No excessive or objectionable laxative effect upon the digestive system of the cows was noted when they were fed the sweet potato meal mixture. The cows fed the sweet potato meal had a sleeker, brighter hair coat than did those fed the corn ration.

#### REFERENCES

- (1) BRANDT, A. E. Test of Significance in Reversal or Switchback Trials. Iowa Agr. Expt. Sta. Bull. 234. 1938.
- (2) BRIGGS, H. M., GALLUP, W. D., HELLER, V. G., DARLOW, A. E., AND CROSS, F. B. The Digestibility of Dried Sweet Potatoes by Steers and Lambs. Okla. Agr. Expt. Sta. Tech. Bull. T-28. 1947.
- (3) COCHRAN, W. G. Modifying Double-reversal Design in Statistically Analyzing Data for Feeding Experiment. Private consultation, Institute of Statistics, Raleigh, N. C. 1946.
- (4) COCHRAN, W. G., AND COX, GERTRUDE M. Experimental Designs. Institute of Statistics, Raleigh, N. C. Mimeograph materials. Pp. 29-30. 1944.



- (5) COPELAND, O. C. Dehydrated Sweet Potato Meal versus Yellow Corn as a Carbohydrate Feed for Lactating Dairy Cows. Tex. Agr. Expt. Sta. Progress Rept. 727. 1941.
- (6) CULLISON, A. E. Dried Sweet Potatoes for Finishing Calves. Miss. Farm Research Bull. 8. 1944.
- (7) DARLOW, A. E., CAMPBELL, W. D., CROSS, F. G., HELLER, V. G., AND BRIGGS, H. M. Feeding and Grazing Experiments with Beef Cattle. Okla. Mimeo. Cir. 136. Pp. 19-21. 1945.
- (8) DARLOW, A. E., CAMPBELL, W. D., CROSS, F. G., HELLER, V. G., AND BRIGGS, H. M. A Comparison of Dried Sweet Potatoes, Wheat, and Corn for Fattening Steer Calves. Okla. Agr. Expt. Sta. Bull. 296. Pp. 39-42. 1946.
- (9) DUNCAN, H. R. Dehydrated Sweet Potatoes versus Corn-cob Shuck Meal. Tenn. Agr. Expt. Sta. Ann. Rept., 54: 28-29. 1942.
- (10) GODBEY, C. G., AND STARKEY, L. U. Sweet Potato Meal for Fattening Hogs in Dry Lot. S. C. Agr. Expt. Sta. Ann. Rept., 55: 71-72. 1942.
- (11) GRIMES, J. E. Sweet Potato Meal as a Substitute for Corn in Rations for Beef Cattle, Mules and Hogs. Ala. Polytech. Inst. Ann. Rept., 52: 15. 1941.
- (12) GRIMES, J. E. Sweet Potatoes and Sweet Potato Products for Fattening Steers. Ala. Polytech. Inst. Ann. Rept., 53: 15. 1942.
- (13) JACOB, M. Beef Cattle Feeding Experiment at Jackson. Tenn. Agr. Expt. Sta. Ann. Rept., 53: 21-22. 1940.
- (14) MASSEY, Z. A. Sweet Potato Meal as a Livestock Feed. Ga. Expt. Sta. Press Bull. 522. 1943.
- (15) RUSOFF, L. L., SEATH, D. M., AND MILLER, G. D. Dehydrated Sweet Potatoes—Their Feeding Value and Digestibility. J. Dairy Sci., 30: 769-774. 1947.
- (16) SEATH, D. M. Dehydrated Sweet Potatoes a Good Feed. La. Agr. Expt. Sta. Ann. Rept. 1942-1943. Pp. 62-63. 1943.
- (17) SEATH, D. M., RUSOFF, L. L., MILLER, G. D., AND BRANTON, C. Utilizing Sweet Potatoes as Feed for Dairy Cattle. La. Agr. Expt. Sta. Bull. 423. 1947.
- (18) SNEDECOR, G. W. Statistical Methods. 4th Ed. The Collegiate Press, Inc., Ames, Iowa. Pp. 421-423. 1946.

## CORRELATION BETWEEN THE LACTOSE CONTENT OF MILK AND THE CEREBROSIDE AND CHOLINE CONTENT OF BRAIN

D. P. SADHU<sup>1</sup>

*Department of Dairy Husbandry, University of Missouri, Columbia*

Although the composition of cerebrosides is well known, their function is not understood. They are found in almost all tissues but are present in largest amounts in the brain. As the cerebrosides are relatively stable under various conditions (14, 15), they are believed to function as basic structural elements. Although the cerebroside composition of different brains has been studied (9, 12, 14, 15), a systematic study of their relation to other lipids has not yet been made.

The cerebrosides have a galactose residue; sphingomyelins have a choline phosphoric acid residue. This led to the belief (1) that the two radicals might be interchangeable, that galactose might act as a choline sparer in the organism. If true, this would throw some light on the functions of lactose in milk, and also on the functions of cerebrosides and sphingomyelins. If there is such sparing of choline by galactose, it may be of significance in lipotropy and related phenomena. This problem was studied by feeding different levels of lactose. However, large amounts of lactose exert an unfavorable effect, apparently by competition with the glucose at the tissue centers where glucose is metabolized. A report (13) appeared on the effect of lactose feeding on the cerebroside content of the rat brain, but not on the change in choline distribution. Therefore, the author has attempted to find out if there is a correlation between the lactose percentage in milk and the cerebroside content of the brain in several species of mammals.

### METHODS

The brains of the various freshly killed animals were weighed, dried in a vacuum oven for 48 hours, pulverized, weighed, and extracted with absolute methanol in a Soxhlet apparatus for 36 hours. The choline in the extract was determined by the method of Glick (6) and the cerebrosides by the method of Brückner (2, 3). Figures 1 and 2 and table 1 show the correlation between choline and galactose in the brain and the lactose in the milk. The effects of age, gestation, and cortical differentiation on the cerebrosides and choline contents of the several brains are presented in tables 2 to 4.

### RESULTS AND DISCUSSION

No definite major function has yet been ascribed to galactose, although many minor functions have been attributed to it (1). Biochemically,

Received for publication December 15, 1947.

<sup>1</sup> India Government scholar.

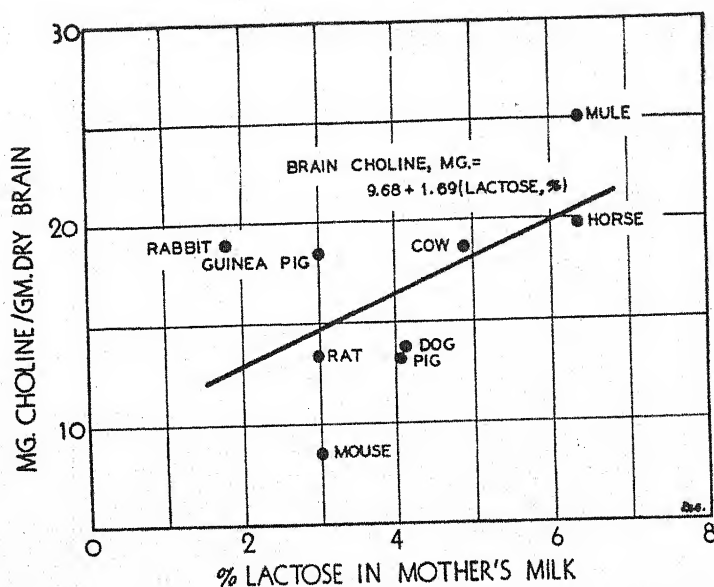


FIG. 1. Correlation of brain galactose with lactose percentage in milk of different species. (Each data point represents one animal.)

galactose is more resistant to oxidation in the body than is glucose. This greater stability of lactose led to the belief that galactoses form a hydrophilic group attached to the sphingosine base. As glucose may form a

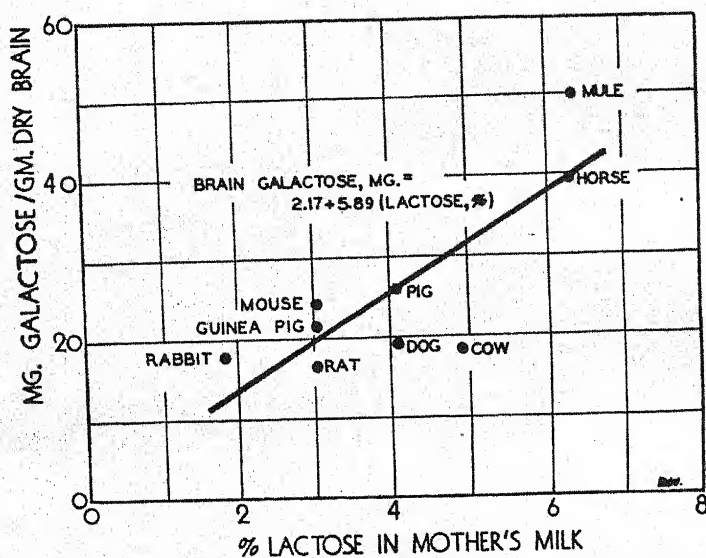


FIG. 2. Correlation of brain choline with lactose percentage in milk of different species.



similar hydrophilic group, gluco-cerebrosides would be expected to exist in the body, and, indeed, gluco-cerebrosides have been isolated from the spleens of patients suffering from Gaucher's disease (5, 7, 11) though the normal galacto-cerebrosides also were present (10). Since then gluco-cerebrosides also have been found in the normal cattle spleen (18) but never in the brain. It is likely that gluco-cerebrosides normally are present side by side with galacto-cerebrosides but, since glucose is the only oxidative substrate for the brain, it is quickly oxidized, and, therefore, not detectable in the brain. Galactose, on the other hand, being resistant to oxidation, forms stable galactosides of brain cerebrosides. This assumption is substantiated by the presence of both gluco- and galacto-cerebrosides in the spleen (8) but only galacto-cerebroside in the brain.

TABLE 1  
*Choline and galactose content of cerebrosides*

Species	No. estimations	Choline (% of dry matter)	Galactose (% of dry matter)
Rat .....	24	1.346	1.667
Mouse .....	12	0.846	2.456
Rabbit .....	18	1.878	1.797
Pig .....	10	1.306	2.621
Guinea pig .....	10	1.831	2.133
Dog .....	6	1.368	1.915
Cow .....	12	1.826	2.239
Horse .....	6	1.979	3.995
Mule .....	10	2.507	5.053

It is true that galactose is synthesized by all animals, but in the mammalian series preformed galactose, supplied by lactose, is likely to determine the galactoside content in the phylogenetic scale of evolution. Feeding of lactose in nontoxic amounts increases galactoside formation in the rat brain (13).

If the galactose can replace the choline phosphoric acid of sphingomyelins, an inverse relationship may be expected between galactose and sphingomyelin contents of brain. Data on rat brain lipids (14, 15) show that as cerebrosides increase with age, sphingomyelins decrease. However, this is true only when these analyses are expressed as percentages of total brain lipids; both cerebrosides and sphingomyelins show an increase in absolute amounts with increasing age. Figure 2 shows that when the choline contents of the brains of different mammals are compared with the lactose contents of milk, a positive correlation is obtained, although a negative correlation was expected. Even if sphingomyelins decrease with increase of galactosides, there may be a simultaneous increase in other choline-containing lipids. As sphingomyelins were not estimated, this question cannot be settled. However, it should be noted, that the correlation between lactose and choline is not very high, and, if a concomitant increase

in lecithins or choline fractions other than sphingomyelins occurs, a negative correlation may be found between lactose and sphingomyelins. It would be interesting to estimate the galactose and sphingomyelin content of the brains of experimental animals fed graded amounts of galactose and choline.

TABLE 2  
*Influence of age on choline and galactose content of brains*

Species	Age	Choline (% of dry matter)	Galactose (% of dry matter)
Rat .....	8 days	2.244	1.385
	14 days	1.718	1.304
	Adult	1.346	1.667
	20 days	1.630	1.180
Mouse .....	Adult	0.846	2.456
	22 days foeti	1.477	1.599
Rabbit .....	Adult	1.878	1.797

TABLE 3  
*Influence of gestation on choline and galactose content of brain*

Species	Choline (% of dry matter)		Galactose (% of dry matter)	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
Rat .....	1.346	1.493	1.667	2.568
Rabbit .....	1.878	1.274	1.797	2.684

TABLE 4  
*Choline and galactose content of cortex and medulla as compared to that of the composite sample of the brain*

Species	Choline (% of dry matter)			Galactose (% of dry matter)		
	Cortex	Medulla	Composite	Cortex	Medulla	Composite
Cow .....	1.755	1.833	1.826	2.185	2.243	2.239
Dog .....	1.337	1.378	1.368	2.053	1.902	1.915

Table 2 shows that there is an increase in the cerebroside content of the brain with age, confirming earlier results (14, 15). Table 3 shows an increase in brain cerebroside with increasing period of gestation, as might be expected, since lactose formation begins in the mother during the gestation period. Table 4 shows no difference in cerebroside content of different species associated with difference in the degrees of cortical differentiation or with different proportions of cortical and medullary tissues.

#### SUMMARY

1. Nine species of mammals have been examined for the cerebroside and choline contents of their brains. Brain cerebroside shows a high positive correlation with the lactose percentage of respective milks. There

is a slight positive correlation between the choline content of brains and the lactose content of the mothers' milk.

It is suggested that sphingosine base can reversibly take up galactose or choline phosphoric acid to form cerebrosides or sphingomyelins and, thus, cerebrosides may function as choline spacers.

2. There is an increase of cerebrosides with age and gestation. There is no significant difference in the cerebroside distribution between cortex and medulla of the brains of the species studied.

## REFERENCES

- (1) BRODY, S., AND SADHU, D. P. The Nutritional Significance of Milk with Special Reference to Milk Sugar. *Sci. Monthly*, 64: 5-13. 1947.
- (2) BRÜCKNER, J. Die Bestimmung der Cerebroside mit Hilfe des Pulfrich-Stufenphotometers. *Z. physiol. Chem.*, 268: 163-170. 1941.
- (3) BRÜCKNER, J. Über den Gehalt des Blutes an Cerebrosiden. *Z. physiol. Chem.*, 268: 251-256. 1941.
- (4) CHARGAFF, E. The Occurrence in Mammalian Tissue of a Lipid Fraction Acting as Inhibitor of Blood Clotting. *Science*, 85: 548-549. 1937.
- (5) DANIELSON, I. S., HALL, C. H., AND EVERETT, M. R. Glucoside Type of Cerebroside in the Spleen in Gaucher's Disease. *Proc. Soc. Exptl. Biol. Med.*, 49: 569-571. 1942.
- (6) GLICK, D. Concerning the Reineckate Method for the Determination of Choline. *J. Biol. Chem.*, 156: 643-651. 1944.
- (7) KLENK, E., AND RENNKAMP, F. Der Zucker im Cerebrosid der Milz bei der Gaucher-Krankheit. *Z. physiol. Chem.*, 272: 280-282. 1942.
- (8) KLENK, E., AND RENNKAMP, F. Über die Ganglioside und Cerebroside der Rindermilz. *Z. physiol. Chem.*, 273: 253-268. 1942.
- (9) LANFRANCHI, F. Determinazione dei Cerebroside nel Tessuto Nervoso di Varie Specie Animali. *Arch. di sci. biol.*, 24: 120-124. 1938.
- (10) LIEB, H., AND GÜNTHER, V. Der Zucker im Cerebrosid der Milz bei der Gaucher-Krankheit. *Z. physiol. Chem.*, 271: 211-213. 1941.
- (11) POLONOVSKI, J. Sur une glucoside-céramide de la rate. *Bull. soc. chim. biol.*, 25: 44-54. 1943.
- (12) SCHMITZ, E. Gehirn und Ernährung. *Biochem. Z.*, 247: 224-245. 1932.
- (13) WHITNAH, C. H., AND CAULFIELD, W. J. A Study of the Formation, Isolation, and Properties of Milk Sugars. *Kan. Agr. Expt. Sta., 7th Bien. Rept.* Pp. 83-85. Dec., 1934.
- (14) WILLIAMS, H. H., GALBRAITH, H., KAUCHER, MILDRED, AND MACY, ICIE G. The Influence of Age and Diet on the Lipid Composition of the Rat. *J. Biol. Chem.*, 161: 463-474. 1945.
- (15) WILLIAMS, H. H., GALBRAITH, H., KAUCHER, MILDRED, MOYER, ELSIE Z., RICHARDS, A. J., AND MACY, ICIE G. The Effect of Growth on the Lipid Composition of Rat Tissues. *J. Biol. Chem.*, 161: 475-484. 1945.





# ESTIMATING THE AMOUNT OF FEED DERIVED FROM PASTURE BY COWS IN THE CONNECTICUT DAIRY HERD IMPROVEMENT ASSOCIATION

G. E. FRICK AND H. D. EATON<sup>1</sup>

*Departments of Agricultural Economics and of Animal Industries,  
University of Connecticut, Storrs*

In many problems of farm management, estimates of the contribution of pasture to total annual feed consumption of dairy cows are desirable. The most common methods for determining pasture yields may be classified broadly under three headings: (a) Yields per acre of pasture based on agronomic data, a procedure which involves clipping, drying, and chemical analysis of the pasture grasses. (b) By quantitative grazing, which measures the weight changes of livestock and involves estimating the yield of pasture from the recorded weight changes. (c) On the basis of milk production records, when total feed requirements are estimated, barn feeds are known, and pasture appears as a residual. The purpose of this paper is to enlarge on the latter method.

## EXPERIMENTAL PROCEDURE

A measure of energetic efficiency of milk production which describes the input-output relationship of milk production was selected. The measure was applied to production and feed consumption records by month of lactation for cows freshening in the winter months when barn feed equaled total feed input. The computed efficiencies of milk production for the various months of lactation were applied to the milk production records for cows freshening in the various months and estimates were made of the total nutrients required by month of lactation. After the total nutrients required were computed by month of freshening and month of lactation, the contribution of pasture was determined by deducting the known barn feeds from the estimated total requirements.

*A measure of energetic efficiency of milk production.* To obtain an estimate of a cow's consumption of total digestible nutrients (T.D.N.), certain measures which describe the cow's ability to convert feed into milk are used. These measures of energetic efficiency of milk production are based on records of milk output, feed intake, weight of cows, and so on.

Various types of dairy-merit indices have been developed. Davis *et al.* (3) proposed a dairy-merit index relating fat-corrected milk (F.C.M.) and weight (W). Brody and Nisbet (2) and Kleiber (7) relate

Received for publication December 31, 1947.

<sup>1</sup> The authors are indebted to Dr. S. Johnson and Dr. R. G. Bressler, Jr., for their helpful suggestions.

milk production to the three-fourths power of body weight. Other indices relating the amount of feed consumption and the amount of milk production, however, are much more applicable to this analysis. Brody (1) has developed a relationship which defines dairy merit as the ratio of milk-energy production to T.D.N. energy consumption. Based on the ratios of 1 lb. of milk to equal 340 calories and 1 lb. of T.D.N. to equal 1,814 calories, the relationship is:

$$\text{Dairy-merit ratio} = \frac{340 \times \text{lb. 4\% F.C.M. produced}}{1,814 \times \text{lb. of T.D.N. consumed}}$$

Brody used this for comparing milk producing capabilities of various cows within breeds and between breeds and also to compare different mammals. He found that dairy merit varied between different mammals and that the larger breeds of dairy cows had a lower dairy merit than the smaller breeds.

*Dairy merit by month of lactation.* Brody's analysis and use of the concept of dairy merit was entirely on an annual basis. In this study his measure is adapted to monthly input-output data in order to develop dairy-merit indices which lead to a determination of the total T.D.N. consumption and the feed derived from pasture. With the established measures of dairy merit by month of lactation, records of milk production may be used to derive monthly feed requirements. Deducting recorded amounts of T.D.N. in barn feed from these required nutrients should give the contribution of pasture.

The records from ten D.H.I.A. herds over a period of 10 years from 1935 through 1944 served as the source for input-output records. A total of 1,200 lactation records from Holstein cows was obtained, with 100 records in each month of freshening. While these records are subject to the usual limitations in applying a single day's performance to estimate an entire month, the records of concentrate feeding were taken on an individual basis and appeared to be reasonably accurate. The records of barn-fed roughages, however, were recorded as the average of several cows or the entire herd. This method of computing roughage consumption would tend, among other things, to obscure any possible differences that may have been associated with the stage of lactation.

The input-output relationships developed from D.H.I.A. data were based only on those months when pasture was unimportant. This was done on an average basis by combining figures for the barn-feeding months from December through March for all months of freshening, and so obtaining average inputs and outputs for each month in the lactation cycle.

#### RESULTS

The dairy-merit ratios for the different months of lactation, calculated according to Brody's formula, are given in table 1. Since feed inputs



do not decline as rapidly as milk production during the lactation period, dairy merit decreases throughout the period. Disregarding the results for the first partial month, dairy merit averaged about 34 per cent during the second month and 20 per cent during the ninth month, an average decline of 2 per cent per month. The tenth through twelfth months of lactation are subject to more rapidly declining milk production and dairy merit, presumably as a result of advancing gestation.

TABLE 1  
*Estimates of dairy merit by months during the lactation period*

Mo. of lactation	Av. lb. of milk per month <sup>a</sup>	Av. lb. of T.D.N. per month <sup>a</sup>	% dairy merit
1	448	195	43.3 <sup>b</sup>
2	1160	635	34.2
3	1048	626	31.3
4	958	614	29.2
5	870	594	27.4
6	799	576	26.0
7	736	558	24.7
8	650	538	22.6
9	557	520	20.1
10	393	490	15.0
11	267	475	10.5
12	162	456	6.7

<sup>a</sup> Milk production has been converted to 4% F.C.M., while feed inputs have been converted to pounds of T.D.N. on the basis of 0.75 per lb. of grain, 0.50 per lb. of hay, and 0.18 per lb. of ensilage.

<sup>b</sup> Based on production and feed for an average of 10.8 days during the month. Since feed inputs were allocated in the ratio of 10.8 to 30, the amount fed after freshening probably is underestimated and the calculated lactational efficiency correspondingly high.

*Estimating required nutrients, and the contribution of pasture.* The dairy-merit ratio defined by Brody may be converted to give directly the amount of feed obtained from pasture. The formula is as follows.

$$\text{Pounds of T.D.N. from pasture} = \frac{340 \times \text{F.C.M.}}{1,814 \times \text{D.M.}} - \text{Pounds of T.D.N. from barn feed}$$

where D.M. represents the previously calculated dairy-merit ratios.

A first attempt at an estimate of pasture will consider the feed equivalent obtained from pasture for cows freshening in the month of January. Table 2 summarizes the computations. These estimates indicate that such cows obtained about 6 per cent of their April feed requirements from pasture, 50 per cent of their May requirements, 74 per cent of their June requirements, and so on. On an annual basis, pasture accounted for 37 per cent of the total feed requirements.

Monthly feed requirements from cows calving in each month of freshening were computed in a manner similar to that for cows freshening in January. Having computed the feed requirements and the breakdown of source of feed, whether from pasture or from barn feeds, it is possible

to combine feed records by month of lactation and by month of freshening. The monthly composite feed records for a herd organization with equal numbers of cows freshening in each of the calendar months may be obtained in this manner. Table 3 summarizes the amounts of recorded nutrients from grain, hay and ensilage, and the total required T.D.N. based on milk production. The amount of pasture was found by deducting the total recorded T.D.N. per day from the total required T.D.N. per day. When the cows are not getting pasture, this figure would be expected to

TABLE 2

*Estimates of monthly feed requirements and nutrients obtained from pasture for cows freshening in January*

Month	Fat-corrected milk per month	Barn-fed T.D.N. per month	Estimated T.D.N. <sup>a</sup>		
			From all sources	From pasture	
	(lb.)	(lb.)	(lb.)	(lb.)	(%)
Jan. <sup>b</sup> .....	457	556	543	- 13 <sup>c</sup>	- 2.4 <sup>d</sup>
Feb. ....	1058	597	580	- 17	- 2.9
March ....	1079	633	646	+ 13	+ 2.0
April ....	951	571	610	+ 39	+ 6.4
May ....	980	332	670	+ 338	+ 50.4
June ....	906	172	653	+ 481	+ 73.7
July ....	825	179	626	+ 447	+ 71.4
Aug. ....	756	181	627	+ 446	+ 71.1
Sept. ....	654	207	610	+ 403	+ 66.1
Oct. ....	536	276	670	+ 394	+ 58.8
Nov. ....	306	435	546	+ 111	+ 20.3
Dec. ....	186	478	520	+ 42	+ 8.1
Total .....	8694	4617	7301	2684	36.8

<sup>a</sup> Using estimated dairy merit from table 1.

<sup>b</sup> Since cows in the sample freshened throughout the entire month, the data applied to an average period of only 10.8 milking days.

<sup>c</sup> Difference between T.D.N. from all sources and barn-fed T.D.N.

<sup>d</sup> Per cent pasture is of total feed requirements.

be zero and any plus or minus amounts would be due to inaccuracy of the method. The fact that these differences are quite small, however, suggests that the errors in the use of this method are not large.

On an annual basis (table 3), considering a herd with equal numbers of cows freshening in each of the calendar months, pasture accounts for 36 per cent of the total T.D.N., grain contributes 27 per cent, hay 21 per cent and ensilage 16 per cent of the total nutrients. The contribution of pasture to total feed requirements of cows freshening in the various months varies from a high of 38 per cent for cows freshening in March to a low of 33 per cent for cows freshening in August. For brevity, tables similar to table 2 are not presented for cows freshening in the months from February through December.

TABLE 3  
*Estimates of nutrients obtained from pasture in different months on 10 D.H.I.A. farms  
in Connecticut, based on 10-year records of production of milk and  
consumption of grain, hay, and ensilage*

Item	Calendar months												Total for year	% of total T.D.N.
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
<i>Milk produced</i>														
4% F.C.M. ....	22.1	22.0	22.5	23.0	25.6	25.9	24.3	24.2	24.1	23.5	21.7	22.0	.....	.....
<i>Total required T.D.N.</i>														
Based on milk production ....	18.2	17.9	18.4	18.9	20.9	21.8	20.1	19.8	20.1	19.7	18.0	18.4	6966.0	.....
<i>T.D.N. furnished &amp; recorded</i>														
Grain .....	5.5	5.9	5.8	5.9	5.1	4.6	4.7	4.7	4.9	5.1	5.4	5.4	1890.0	27.1
Hay .....	7.4	7.6	7.6	7.0	2.6	0.2	0.2	0.2	0.5	1.6	6.4	7.5	1464.0	21.0
Ensilage .....	5.2	5.1	4.7	4.2	1.6	0.3	0.6	0.9	2.1	3.4	4.7	5.3	1143.0	16.4
Total .....	18.1	18.6	18.1	17.1	9.3	5.1	5.5	5.8	7.5	10.1	16.5	18.2	.....	.....
<i>T.D.N. furnished by pasture</i>														
Difference between required and recorded T.D.N. ....	0.1	-0.7	0.3	1.8	11.6	16.7	14.6	14.0	12.6	9.6	1.5	0.2	2469.0	35.5
% of required T.D.N. fur- nished by pasture .....	(0.5)	(-3.9)	(1.6)	9.5	55.5	76.6	72.6	70.7	62.7	48.7	8.3	(1.1)	.....	.....

(av. lb. per day)

(lb.)



## DISCUSSION

There are several limitations to the measure used in this analysis. As stated by Brody, dairy merit varies from cow to cow and is affected by such factors as changes in body weight and size. Since no weight data were available for the D.H.I.A. records used in this analysis, changes in body weight due to overfeeding or loss of flesh as well as to natural growth may have influenced the dairy-merit indices. Dairy merit is affected by age, which includes a joint relationship of increased body size and advanced maturity as related to increased milk production. This relationship is particularly important when working with lactation curves, since the slope of the production curve varies for cows of different ages; therefore, results are affected by the age of the cows in the sample. Dairy merit is a function of feeding levels and not a constant; the curvilinear total input-output curve based on smoothed data as given by Jensen *et al.* (6) would correspond to dairy-merit ratios increasing from 23.0 per cent at 6,000 lb. of 4 per cent F.C.M. output to 25.4 per cent at 8,000 lb. of 4 per cent F.C.M., and then decreasing to 23.6 per cent at 10,000 lb. of 4 per cent F.C.M. Dairy merit varies with environment, especially temperature. Regan and Richardson (9) found that higher temperatures had an adverse effect upon milk production. Some degree of error in the estimation of pasture could be explained on the basis of temperature, since dairy-merit indices were based on the winter months when temperature, as well as the other environmental conditions, was fairly constant.

While the measure under discussion has these limitations, it may prove particularly useful in two cases. First, as shown above, an estimate of the pasture consumption of a herd can be computed. Second, this measure could be used as a means of estimating pasture yields on a seasonal per acre basis. This would provide another measure for estimating pasture consumption on individual farms as well as an additional check on pasture studies involving lactating dairy cows, in which clipping and grazing methods are used. No significant changes would need to be made in the managerial techniques now used in the grazing methods as employed by Hodgson and Shepherd (5) or in the procedures recommended by the Joint Committee of the American Society of Agronomy, American Dairy Science Association and American Society of Animal Production (8). The results, however, would provide another comparison between the clipping (actual growth) and the grazing (actual consumption) methods. Hodgson *et al.* (4) found some divergence in the two methods which might be verified and explained by the use of a third comparison.

## SUMMARY

The residual method was used in order to determine the contribution of pasture to total feed supply for a sample of 1,200 Holstein cows from

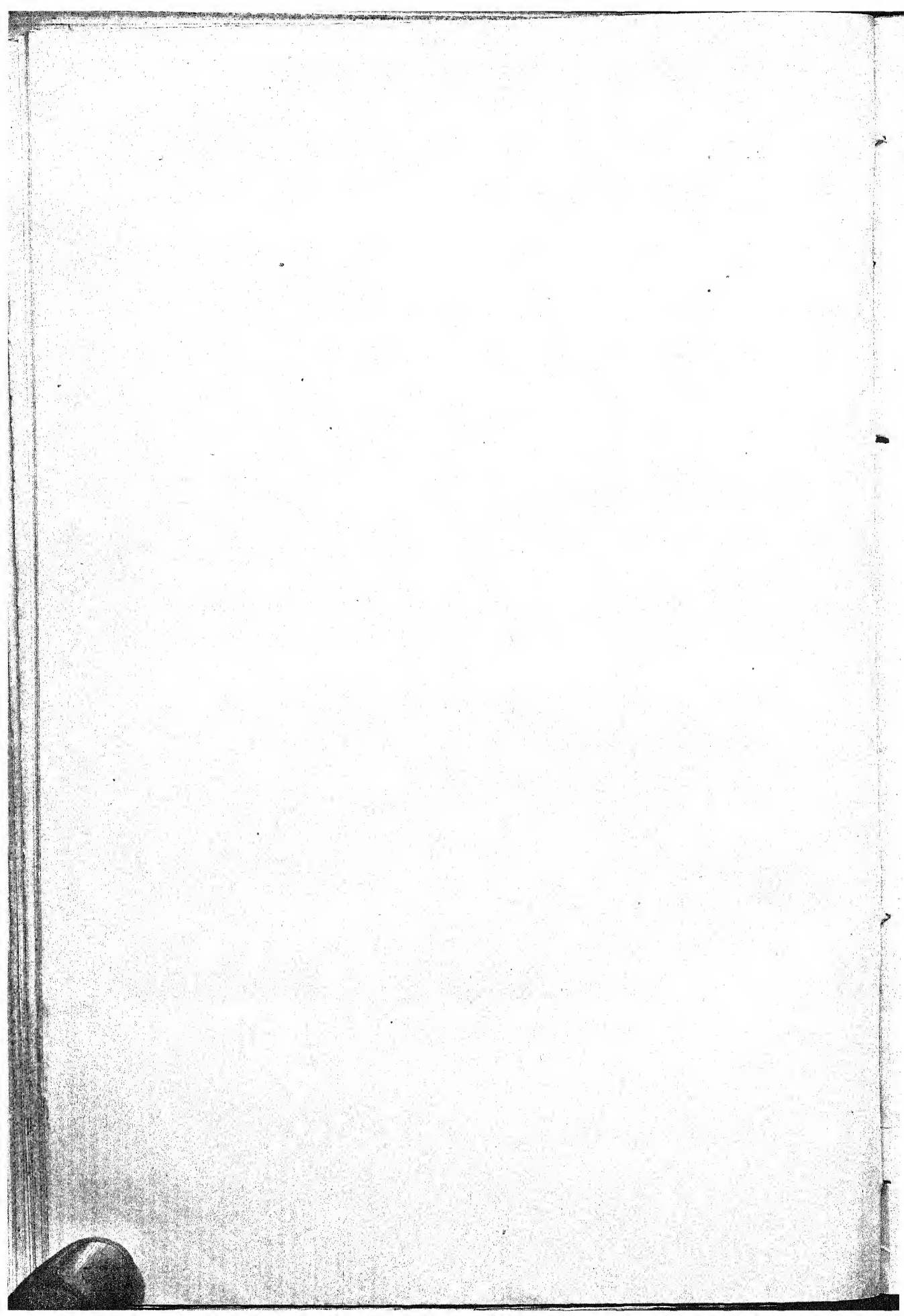
10 D.H.I.A. farms in Connecticut. One of Brody's measures of dairy merit was combined with the input-output records of the 1,200 cows. Dairy-merit indices were computed for each month of lactation on the basis of winter feed and production records. Pasture contributions then were determined by month of lactation for cows freshening in the various months. Pasture accounted for 38 per cent of the total feed for a cow freshening in March and 33 per cent of the feed for a cow freshening in August, on an annual basis. These were the highest and the lowest percentages, respectively.

On a yearly herd basis, using an equal number of cows freshening in each month, pasture accounted for 36 per cent of the total T.D.N. intake, grain for 27 per cent, hay for 21 per cent, and ensilage for 16 per cent of the total nutrients.

While there are limitations, this method may be used in computing pasture yield on a seasonal per acre basis. This would provide an additional check on pasture studies in which clipping and grazing methods are used.

#### REFERENCES

- (1) BRODY, S. Growth and Development. LVI. The Influence of Dairy Merit, Body Size, and Plane of Nutrition on the Economy of Milk Production. Mo. Agr. Expt. Sta. Research Bull. 366. 1943.
- (2) BRODY, S., AND NISBET, R. Growth and Development. XLVII. A Comparison of the Amounts and Energetic Efficiencies of Milk Production in Rat and Dairy Cows. Mo. Agr. Expt. Sta. Research Bull. 285. 1938.
- (3) DAVIS, H. P., MORGAN, R. R., AND GAINES, W. L. Live Weight and Milk-energy Yield in the Nebraska Station Dairy Herd. J. Dairy Sci., 26(7): 625-641. 1943.
- (4) HODGSON, R. E., KNOTT, J. C., MILLER, V. L., AND WOLBERG, F. B. Measuring the Yield of Nutrients of Experimental Pastures. Wash. Agr. Expt. Sta. Bull. 411. 1942.
- (5) HODGSON, R. E., AND SHEPHERD, J. B. A Method for Estimating the Yield of Pastures on Farms in D.H.I.A. J. Dairy Sci., 30(8): 591-592. 1947.
- (6) JENSEN, E., KLEIN, J. W., RAUCHENSTEIN, E., WOODWARD, T. E., AND SMITH, R. H. Input-output Relationships in Milk Production. U. S. Dept. Agr. Tech. Bull. 815. 1942.
- (7) KLEIBER, M. Body Size and Lactation Rate. J. Dairy Sci., 27(8): 593-594. 1944.
- (8) PASTURE IMPROVEMENT COMMITTEE OF THE AMERICAN SOCIETY OF AGRONOMY, COMMITTEE ON PASTURE RESEARCH OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, AND SPECIAL COMMITTEE ON PASTURE AND RANGE INVESTIGATIONS OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION. J. Dairy Sci., 26(4): 353-369. 1943.
- (9) REGAN, W. M., AND RICHARDSON, G. A. Reactions of the Dairy Cow to Changes in Environmental Temperature. J. Dairy Sci., 21(2): 73-79. 1938.





## EFFECT OF WATER SPRINKLING WITH AND WITHOUT AIR MOVEMENT ON COOLING DAIRY COWS

D. M. SEATH<sup>1</sup> AND G. D. MILLER

*Dairy Research Department, Louisiana Agricultural Experiment Station, Baton Rouge*

The knowledge that cattle produce some insensible perspiration yet lack true sweat glands (1) has caused a great deal of speculation among livestock men as to just how much effect artificial sprinkling or wetting by rain may have on cooling cows during warm weather. Likewise, there is much difference of opinion as to how much effect air movement, such as wind, may have on the cooling of cows. This applies to cows prior to sprinkling as well as after sprinkling.

In a previous experiment at this Station (4) it was found that milk cows when removed from the sunshine to shade cooled much faster when sprinkled with water than when not sprinkled. Body temperatures for those sprinkled reduced to normal levels in 1 hour, while those not sprinkled averaged approximately 0.5° F. higher. Also, the reduction in respiration rate was almost twice as great for those sprinkled as for those not sprinkled. Unpublished results of other Louisiana Station experiments (3) have shown that natural rain tends to cool milk cows rapidly. Experimental work in India (2) has shown this same tendency, with the body temperatures of water buffaloes, hill cattle, and sheep dropping considerably and those of zebu cattle showing somewhat less reduction due to heavy natural showers. It was found that wetting animals by hosing for 3 minutes was as effective in cooling water buffaloes as was allowing them to wallow for 20 minutes or for 1 hour.

In a later Indian experiment (5), 15 water buffaloes produced significantly more milk when wetting of their bodies was carried on by splashing. The authors concluded that body wetting was essential for water buffaloes during the hot months.

As reported by Kendall (1), the water lost by insensible perspiration by cattle on a maintenance ration was two to three times greater than that passed in the urine. This loss, he reports, may vary 12 or more lb. per day, with decreases accompanying a drop in air temperature. This large moisture loss, while not true sweat, appears to be relatively important, and the variations normally occurring may be associated with air movement as well as with changes in air temperatures. This experiment was designed to determine the importance of these factors plus others associated with air movement and body sprinkling on the cooling of dairy cows.

Received for publication January 12, 1948.

<sup>1</sup> Now at the University of Kentucky.

## EXPERIMENTAL PROCEDURE

Six Jersey cows in milk were utilized in a  $2 \times 2$  factorial design (6), being divided as equally as possible into two groups of three cows each. Twelve warm clear days between July 11 and August 5, 1947, were selected for conducting this experiment. On each day selected all six cows were tied outside in the sun between 12:00 noon and 2:00 p.m., after which records were made of respiration rates taken from flank movements and of rectal body temperatures. Following this, three of the cows were completely wet by use of a hand sprinkler containing water averaging approximately 85° F. in temperature. Then the cows were put into a special section of the experimental barn. This section was enclosed and rectangular in shape with a 40-inch exhaust fan at one end. Panel-like doors could be opened at the far end when the fan was running to permit circulation of air. The area was divided into three stalls but the sides of the stalls were not solid, thus allowing air circulation. Two cows were tied in

TABLE 1

*Factorial design for determining effectiveness of water sprinkling on cooling dairy cows (6 Jersey cows)*

Test days	Fan operation	Sprinkling procedure <sup>a</sup>	
		Group A	Group B
1, 5, 9	Without fan	Dry	Wet
2, 6, 10	Without fan	Wet	Dry
4, 7, 11	With fan	Wet	Dry
3, 8, 12	With fan	Dry	Wet

<sup>a</sup> 3 cows in each group.

each stall, one at each end, and changes from stall to stall were made systematically from day to day. On half of the days the fan was started as soon as the cows entered the stall and continued running for 1 hour. During the other days the cows remained in the stalls without the fan being in operation. Tests made for wind velocity by use of a Taylor Biram-type anemometer were rather unsatisfactory but showed practically no detectable movement near the floor and from 40 to 240 feet per minute at levels between the cows' bellies and their backs. The division of the cows and treatments is given in table 1.

As shown, each treatment was performed on three different test days. This particular design, as can be seen, has the effects of fan versus no fan confounded with differences caused by variations between test days (6). The effects of treatments as described were determined by noting changes in body temperature and respiration rate after cows had remained 0.5 hour and 1 hour in the special section of the experimental barn.

## RESULTS

*Records on cows while in sun.* After the cows had remained in the

TABLE 2

*Average body temperatures and respiration rates of individual cows after standing in sun for 2 hours*

Cow no.	Body temperature (°F.)		Respiration rate (times per min.)	
	Range	Av.	Range	Av.
X1	102.1-104.7	103.47	80-130	106.3
4	101.5-103.4	102.46	66-115	89.3
X5	101.3-105.1	103.36	92-138	112.5
12	103.8-107.4	105.91	136-175	153.9
X4	101.2-104.0	102.84	75-132	104.9
X7	101.5-104.6	102.92	68-106	86.8
Av.	.....	103.49	.....	109.0

sunshine for 2 hours, the rectal body temperatures (table 2) ranged from 101.2 to 107.4° F. and averaged 103.49° F. The average temperature prior to the cooling treatments thus was approximately 2.0° F. higher than that which usually is considered normal. Respiration rates varied from 66 to 175 per minute, with an average of 109. This average was more than twice as high as were the respiration rates during cool weather.

The variations shown in these preliminary records prior to cooling appear extremely large. Much of this variation was due to differences

TABLE 3

*Comparative average reductions in body temperatures of cows due to wetting and air movement*

Test days	Atmospheric records at 2 p.m.		Decrease in body temperature at end of 1 hr.	
	Temperatures	Humidity	Dry group	Sprinkled group
	(° F.)	(%)	(° F.)	(° F.)
	Without fan			
1	90	36	0.63	0.83
2	92	45	0.77	1.77
5	94	46	0.53	1.07
6	87	29	0.47	1.07
9	93	50	0.70	1.07
10	92	48	1.10	1.77
Av.	91.3	42.3	0.70	1.26
	With fan			
4	92	42	1.47	1.77
3	90	61	1.00	1.43
7	97	47	1.30	1.77
8	94	49	1.33	2.03
11	94	43	1.70	1.90
12	96	44	1.53	2.17
Av.	93.8	47.7	1.39	1.84



between individual cows as well as to the day to day variability found in air temperatures (table 3). Air temperatures ranged from 87 to 97° F. and averaged 91.3° F. on days when the fan was not used and 93.8° F. on the days when the fan was used.

*Influence of cooling on body temperatures.* Statistically significant differences (6) were found in favor of sprinkling as contrasted to not sprinkling and in favor of air movement caused by fan as compared to no fan (tables 3, 4 and 5). Cows left dry without fan showed an average decrease in body temperature after 0.5 hour of 0.28° F. as contrasted to 0.62° F. with fan alone, 0.83° F. for sprinkling alone, and a decrease of 1.42° F. for cows receiving both sprinkling and fan. At the end of 1 hour for the non-sprinkled and non-fan treatment, the decrease in body temperatures averaged only 0.70° F., and for sprinkling plus fan, 1.84° F.

TABLE 4

*Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 0.5 hour*

Cow no.	Av. reduction in body temperature after 0.5 hr.				Av. reduction in respiration rate after 0.5 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° F.)	(° F.)	(° F.)	(° F.)	(times per minute)			
X1	0.93	0.53	0.57	1.30	24.0	35.7	35.3	41.3
4	0.33	0.57	0.73	0.83	25.0	32.0	22.0	52.0
X5	0.33	0.97	0.90	1.23	25.7	30.0	37.7	40.7
12	-0.10	1.70	0.53	2.47	-3.0	64.3	18.0	43.0
X4	0.10	0.57	0.37	1.50	16.7	31.0	26.7	48.0
X7	0.40	0.63	0.63	1.20	10.0	30.7	23.0	29.0
Av.	0.28	0.83	0.62	1.42	16.4	37.3	27.1	42.3

The trend appears to be consistent whether considered for individual test days (table 3) or for individual cows (tables 4 and 5), except in the case of the cows sprinkled without fan; those cows had cooled more at the end of 0.5 hour than had the dry cows with the benefit of fan. This was reversed, however, at the end of 1 hour, when the fan-treated cows were the cooler, thus showing the slower cooling effect produced by the fan alone.

*Influence of cooling on respiration rates.* For cows receiving the sprinkling treatment in this experiment, respiration rates per minute decreased more on an average at the end of 0.5 hour of cooling (table 4) than at the end of 1 hour (table 5). The cows left dry either with or without fan failed to follow this trend, i.e., those without fan decreased an average of 16.4 respirations per minute at the end of 0.5 hour and 20.8 at the end of 1 hour. With fan, the decrease was 27.1 at the end of 0.5 hour and 37.1 at the end of 1 hour. In contrast, those sprinkled decreased

37.3 at the end of 0.5 hour and 31.8 at the end of 1 hour; those with sprinkling and fan reduced 42.3 times per minute in 0.5 hour and 35.3 at the end of 1 hour.

Cow no. 12 (table 4), on an average, failed to decrease either in respiration rate or body temperature at the end of 0.5 hour when left dry and without fan. At the end of 1 hour a slight average reduction in respiration of 3 times per minute was shown, while body temperature also showed an average decrease. This particular cow suffered much from exposure to sunshine and had high body temperatures and respiration rates prior to

TABLE 5

*Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 1 hour*

Cow no.	Av. reduction in body temperature after 1 hr.				Av. reduction in respiration rate after 1 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° F.)	(° F.)	(° F.)	(° F.)	(times per minute)			
X1	1.13	1.20	1.53	1.97	34.3	35.0	44.0	40.3
4	0.43	0.83	1.00	1.27	21.7	31.3	38.7	41.3
X5	0.77	0.93	1.80	1.87	28.0	25.7	47.3	34.0
12	0.77	2.90	1.27	2.70	3.0	64.0	36.7	34.3
X4	0.40	0.80	1.30	1.70	23.3	15.3	29.0	39.7
X7	0.70	0.90	1.43	1.57	14.7	19.7	26.7	22.3
Av.	0.70	1.26	1.39	1.84	20.8	31.8	37.1	35.3

cooling. The fact that she had calved only shortly prior to the start of the experiment partially explains her unusual reactions.

#### DISCUSSION

Results from this experiment give information in addition to that already published (4) concerning the cooling of cows in Louisiana. Of particular interest is the added information that the circulation of air by a fan greatly increases the cooling of both the non-sprinkled and sprinkled cows. For the non-sprinkled cows fanning increased the cooling rate by convection because the ambient air temperature was below that of the body. Perhaps evaporation of insensible perspiration also is a factor, but this is a problem which remains to be investigated. There is need for partitioning the heat loss between convection, vaporization, and radiation, all of which are affected by the vapor pressure of the air and skin and by the air movement.

These trials showed that the least cooling of cows took place without any sprinkling or air circulation and the best results were secured when cows were first sprinkled and then subjected to air circulation. The use of sprinkling alone or fan alone produced essentially equal results, with

sprinkling producing the greater drop in both body temperature and respiration rate at the end of 0.5 hour (table 4) and the fan being the more effective by the end of 1 hour (table 5). In these comparisons it is of interest that sprinkling alone produced a lower respiration rate at the end of 0.5 hour than was present after 1 hour. This was not true for body temperature, which is much slower to respond to cooling treatments. The fact that the cows were sprinkled just once and with water at approximately 85° F. probably helps explain why the maximum reduction in respiration was secured at the end of the 0.5-hour period.

#### SUMMARY AND CONCLUSIONS

When cows were removed from sunshine, sprinkled with water and then subjected to a gentle breeze produced by a fan, they showed rapid changes toward normal body temperature and respiration rate. Shade alone showed a small change in that direction, while the fan alone and sprinkling without a fan were intermediate in their effects.

The results of this experiment give valuable information on how rain and wind as produced by nature tend to cool milking cows during summer months. The results also suggest the need for further experimental work on how mechanical sprayers and fans may be utilized economically during summer when nature is not producing wind or rain.

#### REFERENCES

- (1) KENDALL, J. C. Perspiration a Factor in Animal Metabolism. N. H. Agr. Expt. Sta. Ann. Rept., 1930, Bull. 256, pp. 5-6. 1931.
- (2) MINETT, F. C. Effects of Artificial Showers, Natural Rain and Wallowing on the Body Temperatures of Animals. J. Animal Sci., 6: 35-49. 1947.
- (3) SEATH, D. M., AND MILLER, G. D. Unpublished observations. 1945-1946.
- (4) SEATH, D. M., AND MILLER, G. D. Effect of Shade and Sprinkling with Water on Summer Comfort of Jersey Cows. J. Dairy Sci., 30: 255-261. 1947.
- (5) SINHA, K. C., AND MINETT, F. C. Application of Water to the Body Surface of Water Buffaloes and Its Effect on Milk Yield. J. Animal Sci., 6: 258-264. 1947.
- (6) SNEDECOR, G. W. Statistical Methods. 4th Ed. Collegiate Press, Ames, Iowa. 1946.



## MEASUREMENT OF FLUORESCENT MATERIALS IN MILK AND MILK PRODUCTS<sup>1</sup>

ROBERT JENNESS AND S. T. COULTER

*Divisions of Agricultural Biochemistry and Dairy Husbandry,  
University of Minnesota, St. Paul*

A number of workers in recent years have shown that various dehydrated food products and extracts thereof emit blue fluorescence when subjected to ultraviolet radiation. The intensity of fluorescence increases during storage of the products, and, in some cases, is correlated with loss of palatability, as Fryd and Hanson (8) found with dried eggs. Others, notably at the National Research Council of Canada (28, 36, 40, 41, 42, 43), have demonstrated that heat treatment and storage of dried egg increase the blue fluorescence of a 10 per cent potassium chloride extract (26, 27) of the defatted powder. While Pearce (21) at first postulated that the active fluorescing materials in such extracts are hydrolytic products of the proteins, it appears much more likely that they arise by interaction of the proteins with reducing sugars (1, 13, 20, 25, 33). Furthermore, although Pearce and Thistle (26) and Thistle *et al.* (35) concluded that loss of palatability of dried egg is correlated significantly with increase in fluorescence of the salt-soluble constituents, others (2, 7) have presented evidence indicating a much closer relation between flavor and fluorescence arising in the lipid fraction by reaction of lipid amines with aldehydes (5, 6). Fluorescence has been suggested as an index of storage deterioration of dehydrated pork, dried banana, dried parsnip, ration biscuits, and butter (22) and also for following development of rancidity in lard (10).

Only very meager data are available on the fluorescence of milk, dairy products or milk constituents. It is well known that normal fresh milk emits greenish-yellow fluorescence when irradiated with ultraviolet light (3, 30) and that this fluorescence is due principally to riboflavin (34, 44). Gerngross and Schultz (9) observed that irradiation of milk causes an alteration in fluorescence from yellow to blue, perhaps by conversion of riboflavin to lumichrome (17), although Raoul's (31) data do not substantiate this explanation. Radley (29), finding that severe heat treatment and roller drying of milk shifted its fluorescence to the blue, attributed this change also to a breakdown of riboflavin.

To date the only published quantitative data on the fluorescence of milk other than that due to riboflavin are those of Pearce (22, 23), who studied the salt-soluble fluorescent compounds of defatted<sup>2</sup> dry whole milk.

Received for publication January 20, 1948.

<sup>1</sup> Paper no. 2370, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

<sup>2</sup> It is not clear in the original just what solvent was used to extract the fat or how complete an extraction was attained.

He found: (a) greater initial blue fluorescence in extracts from spray dried than in those from roller dried milks and (b) decided increases upon storage. Since the increase in fluorescence was only slightly correlated with loss of palatability, Pearce (22, 23, 24) has concluded that fluorescence is not a satisfactory index of palatability in milk powder.

The fluorescence of the lipides of milk has not been studied extensively. Morgan and MacLennan (19)<sup>3</sup> found that the fluorescence of butter and butterfat is yellow in contrast to that of margarine, which is blue, and Pearce (22) has reported a decidedly greater blue fluorescence in a butter serum (in 10 per cent potassium chloride solution) from rancid than from fresh butter.<sup>4</sup> This latter observation has been made the basis of a method for assessing deterioration in butter (11).

The fact that several proteins, including casein, exhibit fluorescence when illuminated with ultraviolet has been demonstrated by Reeder and Nelson (32) and also by Vlès (39).

The work reported in this paper represents an attempt to develop a method capable of distinguishing among blue fluorescing materials produced in milk by such reactions as (a) breakdown of riboflavin or other reactions producing soluble blue-fluorescing material (b) lipide-amine-aldehyde interaction, and (c) interaction of protein and sugar. It was hoped that such a method would prove of value in following the course of storage deteriorations of dry milk products and possibly might serve as an objective criterion of palatability changes.

#### METHOD

In attempting to segregate fluorescing compounds from the several possible sources, a standard empirical method was devised involving fractionation of the constituents of milk into four categories: (I) those soluble in 67 per cent acetone; (II) those insoluble in 67 per cent acetone but soluble in acetone-ether (20:80); (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride; and (IV) those insoluble in (I), (II), or (III). This method is applicable to fluid milk or to dry milk reconstituted to a fresh basis. For dry whole milk, a 4-g. sample may be reconstituted by shaking with 31 ml. of distilled water.

The fractionating procedure is as follows: Add two volumes of acetone to one volume of milk or reconstituted milk, mix thoroughly, and filter through 15 cm. paper (S. and S. no. 597) which previously has been extracted exhaustively with 67 per cent acetone (2 acetone + 1 water). Wash the precipitate on the filter with two successive 10-ml. portions of 67 per cent acetone and make the combined filtrate and washings up to 100 ml. with 67 per cent acetone. This constitutes extract I.

<sup>3</sup> See also review by Dérivé (4).

<sup>4</sup> It is not stated whether the sample exhibited hydrolytic or oxidative rancidity.

Grind the precipitated residue from the first extraction with 20 ml. of C.P. acetone, transfer to a 250-ml. Erlenmeyer flask stoppered with a foil-covered cork, shake mechanically for 10 minutes and filter through 15 cm. paper (S. and S. no. 597). Further extract by shaking mechanically for 10 minutes with each of two successive 40-ml. portions of anhydrous ether. The combined acetone-ether filtrates made up to 100 ml. with ether constitute extract II. Although the solvent extracts small amounts of fluorescing material from the filter paper, no appreciable error is introduced if the blank is filtered in the same manner as the sample.

Dry the remaining protein residue by exposure to air at room temperature for 1 hour. Shake a 1-g. sample of it with 25 ml. of 10 per cent potassium chloride solution for 10 minutes and filter, again using S. and S. no. 597, 15 cm. paper. Wash the residue with two successive 25-ml. portions of the 10 per cent potassium chloride solution and make up to 100 ml. with 10 per cent potassium chloride. This is extract III. The 10 per cent potassium chloride does not extract fluorescing materials from the paper.

Extracts prepared in this manner were crystal clear. It will be noted that extract I is essentially identical to the filtrate used by Hand (12) for determination of riboflavin. To determine riboflavin, prepare tubes as follows and measure their fluorescence with the Coleman photofluorometer, using filters *B-2* and *PC-2* (see Hoffer *et al.* (15)).

Reading *A*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. water

“ *B*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. riboflavin solution containing 1 $\gamma$ /ml.

“ *C*—Same as *B* but with fluorescence quenched with approximately 20 mg. of sodium hydrosulfite.

Then:

$$\left( \frac{A - C}{B - A} \right) = \gamma \text{ riboflavin per ml. filtrate}$$

$$\left( \frac{A - C}{B - A} \right) \times \frac{100}{1000} \times \frac{100}{4} = \text{mg. riboflavin per 100 g. powder.}$$

Determine blue fluorescence in each extract with the Coleman photofluorometer using filters *B-1* and *PC-1*. Adjust the galvanometer to read 70 with a quinine sulfate solution containing 0.2 $\gamma$ /ml. Make blank determinations and report results in terms of net galvanometer readings multiplied by any necessary dilution factor.

For fresh whole milk and fresh dry whole milk it usually was necessary to dilute an aliquot of the acetone extract with an equal volume of the 67 per cent acetone solvent in order to obtain a reading on the scale. The ether and potassium chloride extracts from these products gave readings on the scale without dilution. In many aged samples, considerable dilu-



tion of the acetone and potassium chloride extracts was necessary, but rarely was it found necessary to dilute the ether extracts. It appeared desirable to express all results on a common basis and consequently net fluorescence readings were multiplied by dilution factors to convert them to the basis of fluorescence intensity of the 100-ml. extracts. For example,

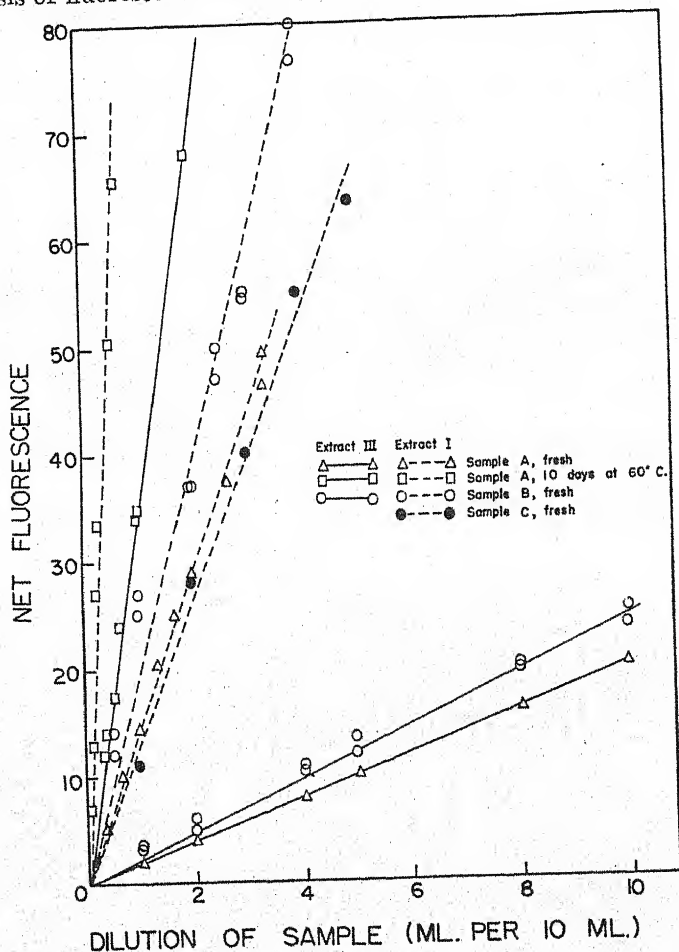


FIG. 1. Relation of fluorescence to dilution of extracts I and III. Indicated aliquots of the extracts were diluted to 10 ml. for fluorescence determination.

a sample diluted by ten times and reading 82.5 with a blank of 31.5 would have a net fluorescence of  $(82.5 - 31.5) \times 10$  or 510. This procedure appears justified by the fact that over the range covered by the instrument the relation between net fluorescence and concentration of the acetone or potassium chloride extracts is linear and passes through the origin, as is illustrated in figure 1.

## MATERIALS

1. *Dried milk* was prepared from University herd milk with the experimental drying equipment of the University of Minnesota.

2. *Casein* was prepared from fresh skim milk by the method of Van Slyke and Baker (38) as modified by Van Slyke (37), with the exception that the extraction with fat solvents was omitted. Calcium phosphocaseinate sols, used as a base for several series of simplified systems, were prepared by dispersing 100 g. of dry casein in 3 l. of saturated limewater, chilling in an ice bath and adding slowly with vigorous agitation 350 ml. of a solution containing 2.98 g. magnesium oxide, 34.47 g. potassium dihydrogen phosphate and 21.95 g. citric acid monohydrate per liter. During this back titration the following materials also were added: 1.014 g. potassium sulfate, 1.260 g. potassium carbonate, 5.600 g. sodium chloride, 1.264 g. potassium chloride, and 3.556 g. calcium hydroxide. The final pH of the sol was 6.7; it was very milky and reasonably stable.

3. *Milk serum protein* was prepared by removal of casein from skim milk with acetate buffer at pH 4.7, followed by exhaustive dialysis of the serum, concentration by pervaporation, freezing, and drying from the frozen state.

4. The *lactose* employed was of USP grade.

5. *Milk fat* usually was obtained by rendering butter and decanting and filtering the fat layer. For one experiment a sample of fat was extracted from whole milk by a macro-adaptation of the Roesse-Gottlieb method. Milk fat was incorporated into the simplified systems by emulsification with a hand homogenizer.

6. The *phospholipide-protein-complex* constituting the so-called "fat globule membrane" was obtained by concentrating and drying from the frozen state the buttermilk and butter serum obtained by churning cream washed by the method of Jenness and Palmer (16).

7. *Riboflavin* was obtained from the Eastman Kodak Company.

8. *Ascorbic acid* was obtained from Hoffmann-LaRoche, Inc.

## EXPERIMENTAL

*Partition of milk constituents among the various fractions.* Some analyses of the several extracts were undertaken to ascertain how the constituents of milk are partitioned by the method used. All of these analyses were made on extracts from whole milk samples, six being dry and one liquid. The data are presented in table I.

Extract I contains 36-43 per cent of the total dry matter of the 4-g. sample. The largest component of this dry matter is lactose; in fact, nearly all of the lactose of the sample appears in this extract. Undoubtedly the failure to recover the lactose quantitatively in extract I is attributable to incomplete washing of the sugar from the precipitate. The lactose de-

terminations were made by evaporating an aliquot of the extract nearly to dryness, making up a definite volume with zinc sulfate and sodium hydroxide solutions according to McDowell (18), filtering and determining lactose in the filtrate by the chloramine-T method of Hinton and Macara (14).

Some nitrogenous material is present in extract I. The amounts of 15.5 to 18.6 mg. per 100 ml. of extract correspond to 46.5–55.8 mg. per 100 g. of milk (12 per cent solids) and hence are somewhat greater than the usual non-protein nitrogen content of milk. Of course extract I also

TABLE 1  
*Analyses of extracts*

Sample	Extract I			Extract II		Extract III
	Dry matter	Nitrogen	Lactose	Dry matter	Lipide P	Protein <sup>a</sup>
	(% of sample)	(mg./100 ml.)	(% of sample)	(% of sample)	(mg./100 g. fat)	(mg./100 ml.)
Dry whole milk						
1	.....	.....	.....	27.0	4.12	.....
2	.....	.....	.....	28.1	4.95	.....
3	40.2	18.2	.....	25.6	.....	74.0
4	42.7	18.6	.....	25.8	.....	72.3
5a	36.4	15.5	29.8 <sup>b</sup>	29.8 <sup>c</sup>	3.10 <sup>c</sup>	62.0
b	.....	.....	.....	28.0	.....	85.7
c	.....	.....	.....	30.6	.....	54.4
6	39.4	.....	31.2 <sup>b</sup>	26.1	.....	.....
Liquid whole milk						
7d	.....	.....	.....	25.5	2.55	83.0

<sup>a</sup> Calculated as total nitrogen  $\times$  6.38.

<sup>b</sup> Lactose content of dry milk determined directly was 32.5 and 35.6% for samples 5a and 6, respectively.

<sup>c</sup> Mojonmier extract of sample 5a yielded 31.2% fat and 27.7 mg. lipide P/100 g. fat.

<sup>d</sup> Calculations made on basis of solids in sample.

contains riboflavin and undoubtedly a number of other minor soluble constituents. It contains very little, if any, fat.

Extract II contains the bulk of the fat of the sample. The amount of fat extracted in this way falls somewhat short of that extracted by the Mojonmier method. The amount of lipide phosphorus in this extract is only a small fraction of that present in the milk and extractable by the Mojonmier method.

The amount of dry matter extracted from 1 g. of defatted protein by the 10 per cent potassium chloride treatment amounts to 175 to 200 mg. The amount of nitrogen extracted by this treatment is equivalent to 54 to 86 mg. of protein. Actually only a portion of this nitrogen represents protein, since it was found that for sample 5c, at least, only approximately



80 per cent of the nitrogen was nondialyzable. Since this extract contains no fat, the non-nitrogenous portion must be composed of minerals and possibly lactose.

The residue that is not dispersed by 10 per cent potassium chloride undoubtedly is largely protein.

*Fluorescence of milk constituents.* The fluorescent characteristics of several milk constituents were studied using the *B-1* and *PC-1* filters in the Coleman instrument. The plot of fluorescence of riboflavin solutions as a function of concentration in figure 2 shows a linear relationship. It is

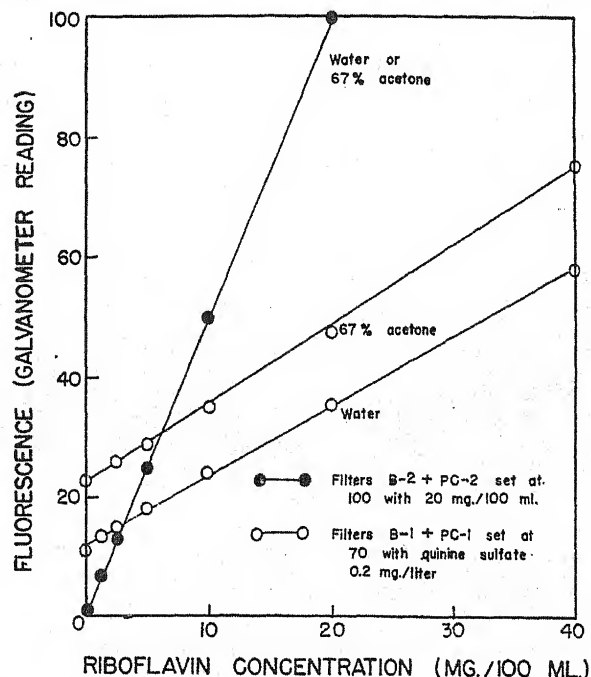


FIG. 2. Fluorescence of riboflavin as a function of concentration.

evident that considerable of the fluorescence of riboflavin is transmitted by filter *PC-1* and consequently that riboflavin accounts for a large portion of the fluorescence of extract I.

In figure 3 the fluorescence of several milk fat solutions is plotted as a function of concentration. The relation is approximately linear. Fat obtained by Roesse-Gottlieb extraction exhibits a much higher fluorescence than that prepared by churning and rendering butter. This comparison between churned and Roesse-Gottlieb extracted fat had to be made with ether as solvent, because the Roesse-Gottlieb extracted fat was not completely soluble in the 20:80 acetone-ether mixture. Undoubtedly this phenomenon, as well as the higher fluorescence of the Roesse-Gottlieb extracted fat, is due to the presence of phospholipides.

The fat obtained in extract II from whole milk exhibits a *net* fluorescence intermediate between those of the Roesse-Gottlieb extracted fats and the churned fats, which is in accord with the previously established fact that extract II contains a portion but not all of the phospholipide of the milk.

The data in table 2 show that both casein and milk serum protein sols in phosphate buffer exhibit blue fluorescence but that the latter fluoresces much more intensely. The fluorescence of milk serum protein preparations may vary considerably, but the fluorescence exhibited by a given

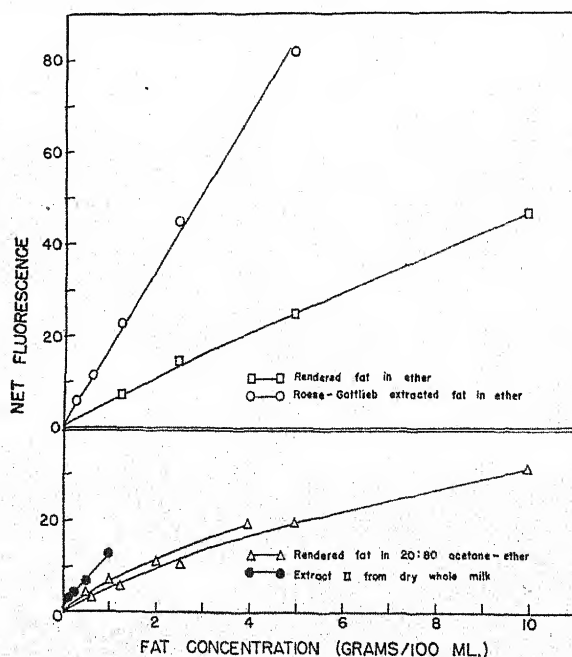


FIG. 3. Comparison of fluorescence of rendered butterfat, extracted fat, and extract II as a function of concentration. Fluorescence of the ether blank was 5.0; that of the 20:80 acetone-ether was 12.0.

preparation in phosphate buffer is approximately identical to that in 10 per cent potassium chloride solution at the same concentration. Casein alone is only slightly dispersible in 10 per cent potassium chloride, but the portion that does dissolve exhibits a considerable fluorescence.

Extraction of the defatted protein fraction of milk by the regular treatment with 10 per cent potassium chloride results in dispersion of only 54-86 mg. of protein from a 1-g. sample, as may be seen in table 1. Furthermore, it was found that grinding of this material with the 10 per cent potassium chloride solvent is without effect on amount of fluorescent material extracted. Variations in the amount of protein extracted do not

TABLE 2  
*Fluorescence of milk protein sols*

Casein sols			Milk serum protein sols		
Sample	Conc.	Net fluorescence	Sample	Conc.	Net fluorescence
	(g./100 ml.)			(g./100 ml.)	
In phosphate at pH 6.6			In phosphate at pH 6.9		
1 .....	0.94	19.0	1 .....	0.54	23.0
2 .....	0.47	10.0	2a .....	0.78	88.0
3 .....	0.23	5.5	2b .....	0.39	48.5
			2c .....	0.20	25.0
Fraction soluble in 10% KCl			In 10% KCl		
Regular <sup>a</sup> .....	0.0063	5.0	2a .....	0.77	Too high
1 grindings <sup>b</sup> .....	0.0207	17.5	2b .....	0.38	49.5
3 grindings <sup>b</sup> .....	0.0344	21.0	2c .....	0.19	24.0

<sup>a</sup> 1-g. samples of casein treated by the method described herein for preparing extract III.

<sup>b</sup> Grinding of casein samples with 10% KCl was used in addition to the shaking employed in the regular method.

appear to be closely related either to the treatment of the product or to the fluorescence of the extract.

Solutions of lactose in water or in phosphate buffer at pH 6.6 and  $\mu = 0.1$  were found to fluoresce only negligibly more than the blank.

*Fluorescence of systems of milk constituents.* In order to obtain further information on the contributions of the various milk constituents to

TABLE 3  
*Fluorescence of simplified systems of milk constituents*

System	Constituents <sup>a</sup>	Net fluorescence						
		Extract I		Extract II		Extract III		
		B <sup>b</sup>	C	B	C	A	B	C
		(per 4 g. complete system)				(per g. protein)		
1	Phosphocaseinate	.....	10.1	.....	.....	.....	.....	8
2	1 + lactose	10.1	16.0	3.8	1.7	3	3	7
3	2 + serum prot.	15.3	25.8	3.5	3.7	4	4	16
4	2 + fat	9.4	17.7	8.5	9.3	3	3	5
5	4 + F.G.M. <sup>c</sup>	17.0	20.7	10.4	9.4	4	5	5
6	5 + serum prot.	24.0	32.0	11.0	10.0	8	6	17
7	6 + riboflavin	51.0	70.0	10.0	10.0	6	6	15
8	7 + ascorbic	51.0	.....	10.0	.....	8	6	.....
Usual value for fresh dry whole milk .....		100.0		11.0		12.0		

<sup>a</sup> Ratio of constituents was as follows: 1.00 casein : 2.15 lactose : 0.30 serum protein: 1.52 fat : 0.04 F.G.M. : 0.000075 riboflavin : 0.0010 ascorbic acid.

<sup>b</sup> Letters designate replicate series.

<sup>c</sup> Fat globule "membrane".



the fluorescence of the several extracts, the standard method described in this paper was applied to a series of simplified systems of milk constituents, each of which was dried from the frozen state.

Table 3 gives the data for fluorescence of these systems, while table 4 gives the contribution of each constituent to the fluorescence. For extracts I and II, fluorescence has been calculated on the basis of 4 g. of the complete system (*i.e.*, the system containing all of the constituents), while for extract III the data are expressed on the basis of 1 g. of protein taken for extraction. The fluorescence of extract I from the system containing all of the constituents mentioned approaches but does not attain that obtained from whole milk. Riboflavin evidently is the major contributor to the fluorescence of extract I, although smaller increments of fluorescent

TABLE 4  
*Contributions of constituents to fluorescence of simplified systems of milk constituents*

Constituent	Contribution to fluorescence						
	Extract I		Extract II		Extract III		
	B	C	B	C	A	B	C
	<i>(per 4 g. complete system)</i>				<i>(per g. protein)</i>		
Caseinate .....	.....	10.1	.....	.....	.....	.....	8
Caseinate + lactose .....	10.1	16.0	3.8	1.7	3	3	7
Serum protein <sup>a</sup> .....	4.2	9.8	-0.3	2.0	1	1	9
Serum protein .....	7.0	11.3	0.6	0.6	4	1	12
Milk fat .....	-0.7	1.7	4.7	7.6	0	0	-2
F.G.M. ....	7.6	3.0	1.9	0.1	1	2	0
Riboflavin .....	27.0	38.0	-1.0	0.0	-2	0	-2
Ascorbic .....	0.0	.....	0.0	.....	2	0	.....

<sup>a</sup> First value given for serum protein is computed as difference between systems 2 and 3, the second as difference between systems 5 and 6.

materials are extracted from the proteins. The serum protein preparation used in series *C* evidently carries more fluorescing materials than that used in series *B*.

Tables 3 and 4 show that the fluorescence of extract II is due principally to the lipides and that the characteristic fluorescence of whole milk extracts is satisfactorily reproduced in systems containing milk fat and fat globule "membrane". The proteins or materials associated with them in the defatted residue are responsible for the fluorescence extractable by 10 per cent potassium chloride from that residue. As expected, fat and riboflavin contribute nothing to the fluorescence of this extract.

*Fluorescence of whole milk and the influence of processing.* The method described herein was applied to 32-ml. samples of liquid whole milk, skim milk and butter milk, all from the same lot, and also to 4-g. samples of dry whole milk, dry skim milk, and dry buttermilk from a second lot. Table 5 indicates that the fluorescence of extract I from these products is approximately equal when expressed on the basis of fat-free solids in the

TABLE 5

*Comparison of fluorescence of extracts from whole milk, skim milk, and buttermilk*

Sample	Riboflavin	Net fluorescence			
		Extract I		Extract II	Extract III
	(mg./100 g. solids)	(per 4 g. solids)	(per 4 g. fat-free solids)	(per 4 g. solids)	(per g. defatted protein)
<i>Series I—Liquid</i>					
Whole milk .....	1.33	108	150	10	11.0
Skim milk .....	2.00	157	157	14	9.0
Buttermilk .....	1.67	147	147	25	17.0
<i>Series II—Dry</i>					
Whole milk .....	1.14	100	139	13	14.5
Skim milk .....	1.47	130	130	7	18.0
Buttermilk .....	1.59	146	146	20	22.2

sample. Buttermilk yielded the most fluorescing materials in extract II, probably because of its higher phospholipide content, but the reason for the high fluorescence of extract III from buttermilk is not apparent. Furthermore, the authors are unable to account for the anomalous relations exhibited by extracts II and III of the skim milk samples in that the liquid and dry samples differ considerably.

The effects of variations in pasteurization temperature, of condensing, and of spray drying were studied on a lot of mixed whole milk. The sample size was adjusted to furnish 4 g. of solids in each case. The results, shown in table 6, indicate that heat treatment in the range of 145 to 195° F.

TABLE 6

*Effect of heat treatment, condensing and drying on fluorescence of extracts*

Sample	Pasteurization temp. for 30 min.	Riboflavin	Net fluorescence		
			Extract I	Extract II	Extract III
	(° F.)	(mg./100 g. solids)	(per 4 g. solids)		(per g. defatted protein)
Whole milk	Raw	0.99	108	12	10.5
" "	145	1.05	116	12	9.5
" "	155	1.05	116	11	11.5
" "	165	1.09	114	14	12.5
" "	175	1.10	124	14	13.0
" "	185	1.10	125	13	13.0
" "	195	1.15	132	13	14.5
Condensed	165	1.22	138	12	9.5
Spray dried	165	1.10	120	12	15.0
Spray dried	150	.....	87	12	7.4
Normal .....	185	.....	95	12	7.4
Spray dried	150	.....	94	12	10.1
High temp. ....	185	.....	93	13	7.4

for 30 minutes has some effect in increasing fluorescence in extract I, possibly some in extract III, but none in extract II. The effects of condensing and spray drying, if any, do not appear to be very marked.

In another experiment, also recorded in table 6, in which pasteurizing treatments of 150 and 185° F. were combined with normal and high temperature drying conditions, no significant effects of either treatment on fluorescence were noted.

#### DISCUSSION

While the procedure adopted for fractionating the constituents of milk is rather empirical, it is felt that a reasonable approach has been made to determining which of those constituents are capable of emitting blue fluorescence when illuminated with ultraviolet. Furthermore, it is considered that the scheme which has been evolved may prove of use in following changes occurring during storage of dry whole milk.

Riboflavin appears to be the principal fluorescent material of extract I but very evidently certain other materials also are involved. In the simplified systems some of the "non-riboflavin fluorescence" was contributed by the protein preparations used, but any speculation on the nature of the specific protein fraction that may be responsible for this effect is fruitless at present. Whole milk evidently contains fluorescent materials soluble in 67 per cent acetone other than those included in the simplified systems, because in no case did the most complete simplified system yield fluorescence in extract I equaling that of whole milk.

The fluorescence of extract II appears to depend, in part, on its phospholipide content. Evidently the major portion of these lipides is not extracted at all but remains in the final residue. Consequently, variations in the fluorescence of this extract might be expected to depend on the extent of extraction of phospholipides. The most satisfactory scheme would be one which extracted all of the phospholipides, but under the empirical conditions used, the proportion of phospholipide extracted probably is sufficiently constant so that no great variations in fluorescence are introduced from that cause.

Ten per cent potassium chloride disperses a fraction of milk protein which is associated with a certain amount of fluorescing ability. No exhaustive attempt was made to determine just which protein fraction is involved. However, the data secured on fluorescence of milk proteins in 10 per cent potassium chloride indicate that both casein and at least some of the serum protein fractions are involved. Here again it might be argued that the ideal situation would be to disperse all of the protein in extract III, leaving no undispersed residue, but to date it has not proved possible to prepare such a dispersion that is satisfactory for fluorescence measurements.

The data fail to indicate any pronounced change in fluorescence proper-



ties during the processing involved in manufacture of dry whole milk. The procedure employed in this study is being applied to following the changes occurring during heat treatment of milk and storage of dry whole milk.

#### SUMMARY AND CONCLUSIONS

A method is presented for evaluating the fluorescence characteristics of milk by fractionating the constituents of milk into (I) those materials soluble in 67 per cent acetone, (II) those insoluble in (I) but soluble in 20:80 acetone-ether, (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride, and (IV) those insoluble in (I), (II) or (III), and determining the blue fluorescence of the solutions of (I), (II) and (III).

As might be expected, riboflavin is the largest contributor to the fluorescence of extract I, although proteins and probably other constituents also contribute. The lipides, particularly the phospholipides, are the fluorescent compounds of extract II, while the proteins contribute the fluorescent materials dispersible in 10 per cent potassium chloride.

The normal processing of dry whole milk appears to be without effect on the fluorescence of these extracts.

#### ACKNOWLEDGMENTS

The research which this paper reports was undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

#### REFERENCES

- (1) BATE-SMITH, E. C., AND HAWTHORNE, J. R. Dried Egg. X. The Nature of the Reaction Leading to Loss of Solubility of Dried Egg Products. *J. Soc. Chem. Ind.*, 64: 297-302. 1945.
- (2) BOGGS, M. M., DUTTON, H. J., EDWARDS, B. G., AND FEVOLD, H. L. Dehydrated Egg Powders. Relation of Lipide and Salt-water Fluorescence Values to Palatability. *Ind. Eng. Chem.*, 38: 1082-1084. 1946.
- (3) DÉRIBÉRÉ, M. La Fluorescence du Lait. *Lait*, 22: 122-134. 1942.
- (4) DÉRIBÉRÉ, M. La Fluorescence du Beurre. *Lait*, 22: 323-327. 1942.
- (5) DUTTON, H. J., AND EDWARDS, B. G. Changes in Stored Dried Eggs. Spectrophotometric and Fluorometric Measurement of Changes in Lipides. *Ind. Eng. Chem.*, 37: 1123-1126. 1945.
- (6) EDWARDS, B. G., AND DUTTON, H. J. Changes in Stored Dried Eggs. Role of Phospholipides and Aldehydes in Discoloration. *Ind. Eng. Chem.*, 37: 1121-1122. 1945.
- (7) FEVOLD, H. L., EDWARDS, B. G., DIMICK, A. L., AND BOGGS, M. M. Dehydrated Egg Powders. Sources of Off-flavors Developed during Storage. *Ind. Eng. Chem.*, 38: 1079-1082. 1946.

- (8) FRYD, C. F. M., AND HANSON, S. W. F. Spray Dried Egg. The Relation between Flavor and Physical and Chemical Characteristics. *J. Soc. Chem. Ind.*, 63: 3-6. 1944.
- (9) GERNGROSS, O., AND SCHULTZ, M. Über die Fluoreszenzerscheinungen von Milch und Milchprodukten im filtrierte Ultraviolettlicht. *Milchw. Forsch.*, 6: 567-574. 1928.
- (10) GRANT, G. A., AND LIPS, H. J. A Study of Methods for Assessing Rancidity in Lard. *Can. J. Research*, 24F: 450-460. 1946.
- (11) GRANT, G. A., AND WHITE, W. H. A Fluorescence Method for Assessing the Keeping Quality of Butter. *Can. J. Research*, 24F: 461-466. 1946.
- (12) HAND, D. B. Determination of Riboflavin in Milk by Photoelectric Fluorescence Measurements. *Ind. Eng. Chem., Anal. Ed.*, 11: 306-309. 1939.
- (13) HAWTHORNE, J. R., AND BROOKS, J. Dried Egg. VIII. Removal of the Sugar of Egg Pulp before Drying. A Method of Improving the Storage Life of Spray-dried Whole Egg. *J. Soc. Chem. Ind.*, 63: 232-234. 1944.
- (14) HINTON, C. L., AND MACARA, T. The Determination of Aldose Sugars by Means of Chloramine-T, with Special Reference to the Analysis of Milk Products. *Analyst*, 52: 668-688. 1927.
- (15) HOFFER, A., ALCOCK, A. W., AND GEDDES, W. F. A Rapid Method for the Determination of Riboflavin in Wheat and Wheat Products. *Cereal Chem.*, 21: 524-533. 1944.
- (16) JENNESS, R., AND PALMER, L. S. Substances Adsorbed on the Fat Globules in Cream and Their Relation to Churning. V. Composition of the "Membrane" and Distribution of the Adsorbed Substances in Churning. *J. Dairy Sci.*, 28: 611-623. 1945.
- (17) KARRER, P., SALOMON, H., SCHÖPP, K., SCHLITTLER, E., AND FRITZSCHE, H. Ein neues Bestrahlungsprodukt des Lactoflavins. *Lumichrom. Helv. Chim. Acta.*, 17: 1010-1013. 1934.
- (18) McDOWELL, A. K. R. The Estimation of Lactose in Milk. *J. Dairy Research*, 12: 131-138. 1941.
- (19) MORGAN, R. S., AND MACLENNAN, K. The Fluorescence of Some Vitamin A Containing Fats. *Biochem. J.*, 22: 1514-1522. 1928.
- (20) OLCOTT, H. S., AND DUTTON, H. J. Changes in Stored Dried Eggs. Source of Fluorescence. *Ind. Eng. Chem.*, 37: 1119-1121. 1945.
- (21) PEARCE, J. A. Behaviour and Nature of the Fluorescing Substances in Dried Egg Powders. *Can. J. Research*, 21D: 98-107. 1943.
- (22) PEARCE, J. A. Fluorescence Development in Various Food Products. *Can. J. Research*, 22F: 87-95. 1944.
- (23) PEARCE, J. A. Dried Milk Powder. I. Methods of Assessing Quality and Some Effects of Heat Treatment. *Can. J. Research*, 23F: 177-184. 1945.
- (24) PEARCE, J. A. Fluorescence Aids in Studying the Quality of Dairy Products. *Can. Dairy Ice Cream J.*, 25: 19-21. 1946.
- (25) PEARCE, J. A., AND BRYCE, W. A. Fluorescence Spectra of Extracts of Dried Whole Egg Powder. *Food Technol.*, 1: 310-320. 1947.
- (26) PEARCE, J. A., AND THISTLE, M. W. Fluorescence as a Measurement of Quality in Dried Whole Egg Powder. *Can. J. Research*, 20D: 276-282. 1942.
- (27) PEARCE, J. A., THISTLE, M. W., AND REID, M. Dried Whole Egg Powder. VIII. An Improved Fluorescence Method and Some Factors Affecting the Measurement. *Can. J. Research*, 21D: 341-347. 1943.
- (28) PEARCE, J. A., WOODCOCK, A. H., AND GIBBONS, N. E. Dried Whole Egg Powder. X. The Effect of Added Substances on Keeping Quality. *Can. J. Research*, 22F: 34-38. 1944.

- (29) RADLEY, J. A. The Fluorescence of Milk and Dairy Products. *Analyst*, 58: 527-529. 1933.
- (30) RADLEY, J. A., AND GRANT, J. Fluorescence Analysis by Ultraviolet Light. Chapman and Hall, London. 1933.
- (31) RAOUL, Y. Recherches Biochimiques sur les Avitaminoses du Groupe de la Pellagre. III. Répartition et Rôle Eventuel de Certain Corps à Fluorescence Bleue. *Bull. soc. chim. biol.*, 25: 279-286. 1943.
- (32) REEDER, W., AND NELSON, V. E. Studies on Fluorescence Associated with Proteins. *Proc. Soc. Exptl. Biol. Med.*, 45: 792-794. 1940.
- (33) STEWART, G. F., BEST, L. R., AND LOWE, B. A study of Some Factors Affecting the Storage Changes in Spray-dried Egg Products. *Proc. Inst. Food. Technol.*, 1943: 77-89. 1943.
- (34) SUPPLEE, G. C., ANSBACHER, S., FLANIGAN, G. E., AND HANFORD, Z. M. The Fluorometric Estimation of Lactoflavin. *J. Dairy Sci.*, 19: 215-220. 1936.
- (35) THISTLE, M. W., PEARCE, J. A., AND GIBBONS, N. E. Dried Whole Egg Powder. I. Methods of Assessing Quality. *Can. J. Research*, 21D: 1-7. 1943.
- (36) THISTLE, M. W., WHITE, W. H., REID, M., AND WOODCOCK, A. H. Dried Whole Egg Powder. XIV. Effects of Low Temperature, Low Moisture Content, Carbon Dioxide Pack, and Copper Contamination on Keeping Quality. *Can. J. Research*, 22F: 80-86. 1944.
- (37) VAN SLYKE, L. L. Chemistry of Casein. *Chem. Age*, 32: 163-165. 1924.
- (38) VAN SLYKE, L. L., AND BAKER, J. C. The Preparation of Pure Casein. *J. Biol. Chem.*, 35: 127-136. 1918. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 65. 1918.
- (39) VLÉS, F. Excitation of Fluorescence of Proteins. *Arch. phys. biol.*, 16: 137-166. 1943.
- (40) WHITE, W. H., AND THISTLE, M. W. Dried Whole Egg Powder. II. Effect of Heat Treatment on Quality. *Can. J. Research*, 21D: 194-202. 1943.
- (41) WHITE, W. H., AND THISTLE, M. W. Dried Whole Egg Powder. IV. Effect of Moisture on Keeping Quality. *Can. J. Research*, 21D: 211-222. 1943.
- (42) WHITE, W. H., THISTLE, M. W., AND REID, M. Dried Whole Egg Powder. VI. Effect of Storage Temperature and Gas Packing on Keeping Quality. *Can. J. Research*, 21D: 271-276. 1943.
- (43) WOODCOCK, A. H., AND REID, M. Dried Whole Egg Powder. IX. Effect of Drying Conditions on Quality. *Can. J. Research*, 21D: 389-393. 1943.
- (44) WURSTER, K. Zur Kenntnis der Fluoreszenz der Kuhmilch im Filtrierten Ultraviolettlicht. *Milchw. Forsch.*, 11: 379-382. 1931.





## THE RELATIONSHIP OF THE CHANGE IN pH EFFECTED BY INCUBATION TO OTHER SEMEN CHARACTERISTICS<sup>1</sup>

J. T. REID,<sup>2</sup> G. M. WARD, AND R. L. SALSBUURY  
*New Jersey Agricultural Experiment Station, Sussex*

A number of simple, rapid tests have been applied to the evaluation of semen, although no single measure is presently recognized as an adequate index of fertilizing capacity. The reports of several workers (1, 2, 4, 5, 8, 15, 17) would indicate that the initial pH of semen may be related to semen quality and/or used as a criterion of relative fertility. Significant correlations have been found between the initial pH and spermatozoa concentration (1, 2, 3, 9, 14, 15, 17), ejaculate volume (1, 2, 9, 17), spermatozoa motility (1, 2, 6, 9, 11, 17), sugar content (5) and buffer capacity (4) of semen, and the glucose loss, lactic acid gain and viability following incubation at 46.5° C. for 1 hour (17). The initial pH was inversely related to these semen characteristics. However, Swanson and Herman (16) found no appreciable relationship between the pH of fresh semen and conception rate.

Although the initial pH was found to be helpful in the evaluation of semen in a number of studies, several reports would indicate that the final pH and/or the change in pH effected by incubation and/or storage of semen provides a more satisfactory index (6, 11, 13, 19, 20). The final pH of semen following incubation at 37° C. and storage at 40° F. was correlated with the concentration, motility, oxygen consumption and fertility of spermatozoa (13) and the conception rate (11), respectively. Although Anderson (4) found no significant correlation between the change in pH upon incubating semen at 37° C. for 1 hour and the buffer capacity or specific gravity of semen, a high correlation was found between the change in pH and the spermatozoa concentration (6, 13), initial motility (6, 13), oxygen consumption (13) and fertility (13, 19, 20, 21) of spermatozoa. Measuring the drop in pH of semen stored at 27 to 29° C. and at 10° C., Dougherty and Ewalt (10) found the decrease in pH to be related to the motility and viability of spermatozoa. These workers showed that refrigeration and chemical inactivation, both of which inhibit motility, also prevent rapid decreases in pH. Several workers (6, 13) believe that the change in pH of semen is a reflection of the general metabolism of spermatozoa involving the number and activity of spermatozoa, respiration and glycolysis. For development of the maximum increase in acidity during

Received for publication January 21, 1948.

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Industry.

<sup>2</sup> Present address, Cornell University, Ithaca, New York.

incubation, Anderson (6) suggested the necessity of a high concentration of actively motile spermatozoa, an adequate quantity of glucose or other reducible sugar, and conditions favorable for the retention in the semen of acid products, *i.e.*, anaerobic conditions.

The present study was initiated when it was observed that, of a large number of semen quality tests employed in this laboratory, the change in pH during incubation seemed to be giving the best over-all estimate of semen quality. Since most of the data recorded in the literature represent a relatively limited number of ejaculates and since the relationships of the pH change to only a few semen characteristics are reported, the purpose of this investigation was to contribute additional data to the subject and to determine the value of the change in pH effected by incubation as an index of semen quality.

#### EXPERIMENTAL PROCEDURE

The relationship of the change in pH upon incubation of semen at 37° C. for 1 hour to concentration, initial motility, and viability of spermatozoa and percentage of abnormal spermatozoa, was studied in 203 semen specimens from 11 bulls. The average sample consisted of 2.33 ejaculates (range, 1-5 ejaculates).

Soon after the semen was ejaculated, the initial motility was estimated by using a constant temperature stage incubator adjusted to 100° F., the initial pH was measured with a Beckman glass electrode potentiometer, and an aliquot of each sample was incubated at 37° C. for 1 hour, after which time the pH was measured again. The spermatozoa concentration was determined with a cytometer, and the percentage of morphologically abnormal spermatozoa was estimated in fixed and stained semen smears. The initial motility ratings used in this study represent gradations of 1 to 20 units and are approximately equivalent to per cent motility divided by five. Viability was measured as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

#### RESULTS AND DISCUSSION

The pertinent data obtained in this study are shown in table 1. Highly significant positive correlations were found between the pH change of incubated semen and each of the characteristics, of concentration, initial motility, and viability of spermatozoa, whereas a significant negative correlation existed between the pH drop and the percentage of morphologically abnormal spermatozoa. Of these semen characteristics, the concentration and initial motility of spermatozoa showed the highest degree of relationship to the decrease in pH. These correlation coefficients (0.460 and 0.436, respectively) are remarkably similar to those reported by Anderson (6) (0.476 and 0.450, respectively). From a casual observation, these coeffi-



TABLE 1  
Correlation coefficients showing interrelationships of various semen characteristics

Semen characteristics	1 pH change	2 Viability	3 Spermatozoa concentration	4 Initial motility	5 Abnormal spermatozoa
1 pH change	.....				
2 Viability	.....	0.2062 ± 0.0675**			
3 Spermatozoa concentration	.....		0.4600 ± 0.0556**		
4 Initial motility	.....		0.2639 ± 0.0636**	0.4364 ± 0.0571**	
5 Abnormal spermatozoa	.....			0.2977 ± 0.0669**	-0.0276 ± 0.0705
Means ± standard error	0.336 ± 0.016 <sup>a</sup>	35.29 ± 2.32 <sup>b</sup>	0.993 ± 0.023 <sup>c</sup>	0.3464 ± 0.0621**	-0.2489 ± 0.0662**
				13.1 ± 0.345 <sup>d</sup>	-0.3557 ± 0.0616**
					13.7 ± 0.680 <sup>e</sup>
Range	0.0-1.64	0.0-128 <sup>f</sup>	0.21-2.04	0.1-19.8	2.0-43.0
$R_{1,2,3,4,5} = 0.5658 \pm 0.0484$					

<sup>a</sup> Units of pH.

<sup>b</sup> Percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

<sup>c</sup> Millions per mm.<sup>3</sup>

<sup>d</sup> Motility value x 5 is approximately equivalent to per cent motility.

<sup>e</sup> Per cent morphologically abnormal.

<sup>f</sup> Three values found above 100%; 12 values above 88%.

\*\* Highly significant.

cients may appear to be low. However, they were calculated from the ungrouped data of individual semen samples.

The multiple correlation coefficient ( $0.5658 \pm 0.0484$ ) between the pH change and the four semen characteristics studied was highly significant. The pH change of incubated semen would seem to have great potential worth in the evaluation of semen, since it is related to a number of different characteristics.

It long has been known that lactic acid formation occurs during the storage of semen and is accompanied by a decrease in the quantity of glucose or other reducing sugars, a reduction in motility and an increase in acidity (18). The incubation of semen increases the rate of these reactions, allowing measurable differences in the end-products within short periods of time. Since the rate and the extent of the decrease in pH were found to be proportional to both motility and concentration of spermatozoa in this study as well as in others (6, 13), the pH drop effected by incubation appeared to be a quantitative reaction dependent upon the metabolic activity of the individual spermatozoan and the total number of spermatozoa present. This provided an indirect measure of the over-all metabolism of semen. That the effects of the percentage of morphologically abnormal spermatozoa reflect upon the change in pH of a semen specimen is indicated by the inverse relationship between these two characteristics (correlation coefficient =  $-0.324$ ). This phenomenon, in which a large number of morphologically abnormal spermatozoa minimized the extent to which pH was changed, would suggest that these spermatozoa are participating in katabolism either very little or not at all. Since deformed spermatozoa tend to impede the motility of the more normal cells, they perhaps further inhibit the rate of metabolism of the total specimen, producing less lactic acid and other acid products, thereby resulting in a lesser degree of pH drop. Table 1 shows a highly significant inverse relationship between the percentage of morphologically abnormal spermatozoa and the initial motility. The abnormal spermatozoa commonly appear to survive the normal spermatozoa, as determined by the maintenance of motility. This observation would indicate the possibility of energy conservation in abnormal spermatozoa and would be in accord with the observations previously discussed. Some studies suggest that results of measures of spermatozoa metabolism, such as the rate of respiration (18) and the rate of glycolysis (7), provide the best single indications of fertility. However, the measures of oxygen consumption and products of glycolysis of spermatozoa are tedious, necessitate special apparatus and conditions restricted to well-equipped laboratories, and require personnel of specialized training, thereby limiting their field usage. A high degree of relationship exists between the pH change and oxygen consumption (13), a number of other semen characteristics and fertility (13, 19, 20, 21). It would seem that complex,

time consuming tests and the use of a large number of simple semen quality tests would be largely obviated by the use of the pH change upon incubation as a single measure of semen quality. Although a pH-meter was employed in this study, a series of indicators could be readily adapted to field use as suggested by Laing (13). Incubation of semen for 1 hour produces greater changes in semen pH than does incubation for 0.5 hour; however, some artificial breeding units may find the shorter incubation period more compatible with their semen collection schedule. Information provided by this test would be available for the inseminator when needed, *i.e.*, before dilution of the semen and before insemination of the cow.

#### SUMMARY

1. A study was made of the relationship of the decrease in pH during incubation of 203 semen specimens composed of 473 ejaculates from 11 bulls to other semen characteristics.

2. Highly significant coefficients of correlation found between the change in pH and other characteristics of semen were: concentration of spermatozoa, 0.46; initial motility of spermatozoa, 0.44; viability of spermatozoa, 0.21; and percentage of morphologically abnormal spermatozoa, -0.32. The multiple coefficient of correlation (0.57) between these four semen characteristics and the drop in pH was highly significant. These data suggest a significant relationship of the pH change in incubated semen to a number of different semen characteristics and indicate its value as an indirect measure of over-all semen metabolism.

3. The results of this study, in combination with those of other investigations involving fertility data, would seem to indicate that the change in pH effected by incubation is probably the best simple, quick test of semen quality available at the present time.

The authors are indebted to Prof. C. E. Shuart and Messrs. Martin Struble and D. Eby for the feeding and management of the bulls and many other services.

#### REFERENCES

- (1) ANDERSON, J. The Hydrogen-ion Concentration of the Semen of the Bull. *J. Agr. Sci.*, 32: 298-307. 1942.
- (2) ANDERSON, J. The Clinical Significance of the Hydrogen-ion Concentration of the Semen of the Bull. *Vet. Record*, 54: 317-318. 1942.
- (3) ANDERSON, J. The Change in the pH of the Semen of the Bull after Incubation. *J. Agr. Sci.*, 34: 69-72. 1944.
- (4) ANDERSON, J. The Buffer Capacity and the Specific Gravity of Bull Semen. *J. Agr. Sci.*, 36: 258-259. 1946.
- (5) ANDERSON, J. The Sugar Content of Bull Semen and Its Reduction during Storage. *J. Agr. Sci.*, 36: 260-262. 1946.
- (6) ANDERSON, J. Some Factors Affecting the pH Change in Bull Semen. *J. Agr. Research*, 37: 11-16. 1947.



- (7) COMSTOCK, R. E., GREEN, W. W., WINTERS, L. M., AND NORDSKOG, A. W. Studies of Semen and Semen Production. Minn. Agr. Expt. Sta. Tech. Bull. 162. 1943.
- (8) DAVIS, H. P. Some Factors Affecting Artificial Insemination in Cattle. Proc. Am. Soc. Animal Production, 31: 246-253. 1938.
- (9) DAVIS, H. P., AND WILLIAMS, N. K. Evaluating Bovine Semen. I. Influence of the Number of Ejaculates upon Various Physical and Chemical Characteristics and the Relationship between Those Factors. Proc. Am. Soc. Animal Production, 32: 232-242. 1939.
- (10) DOUGHERTY, R. W., AND EWALT, H. P. Semen Studies in the Bull. Am. J. Vet. Research, 2: 419-426. 1941.
- (11) ELLENBERGER, H. B., AND LOHMANN, A. H. Artificial Insemination of Dairy Cattle. Vt. Agr. Expt. Sta. Bull. 533. 1946.
- (12) HERMAN, H. A., AND SWANSON, E. W. Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination. Mo. Agr. Expt. Sta. Research Bull. 326. 1941.
- (13) LAING, J. A. Observations on the Characteristics of the Semen in Relation to Fertility in the Bull. J. Agr. Sci., 35: 1-24. 1945.
- (14) RIMOLDI, A., AND BRIGATTI, C. Variations in the pH of Bull Semen in Relation to Sexual Activity and to Individual Characteristics. (Translated title.) Fecond. Artif. Milan. No. 8. 1941. (Abs., Nuova Vet., 20: 214. 1942; Animal Breeding Abstracts, 13: 88. 1945.)
- (15) SCHNEERSON, S. S. The Importance of Determining the pH of Sperm for Artificial Insemination of Cattle. (Translated title.) Sborn. Trud. zooteh, Kaf. s. h. Skol. Kirov., 1: 142-147. 1936. (Abs., Animal Breeding Abstracts, 4: 419-420. 1936.)
- (16) SWANSON, E. W., AND HERMAN, H. A. The Correlation between Some Characteristics of Dairy Bull Semen and Conception Rate. J. Dairy Sci., 27: 297-301. 1944.
- (17) VAN DEMARK, N. L., MERCIER, E., AND SALISBURY, G. W. The Methylene-blue Reduction Test and Its Relation to Other Measures of Quality in Bull Semen. J. Dairy Sci., 28: 121-128. 1945.
- (18) WALTON, A., AND EDWARDS, J. Criteria of Fertility in the Bull. I. The Exhaustion Test. Proc. Am. Soc. Animal Production, 31: 254-259. 1938.
- (19) WEBSTER, W. M. Proc. 7th Ann. Meeting of Sheep Farmers, Massey Agr. Coll., New Zealand. 1938. (Cited by Laing (13).)
- (20) WEBSTER, W. M. Proc. 8th Ann. Meeting of Sheep Farmers, Massey Agr. Coll., New Zealand. 1939. (Cited by Laing (13).)
- (21) WEBSTER, W. M. Personal communication. (Cited by Laing (13) and Anderson (2).)

ASSOCIATION ANNOUNCEMENTS  
PROGRAM  
FORTY-THIRD ANNUAL MEETING  
OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF GEORGIA  
ATHENS, GEORGIA  
JUNE 14-16, 1948

PROGRAM COMMITTEE

GENERAL:

FORDYCE ELY, *Chairman*  
Ohio State University  
JENNINGS B. FRYE  
University of Georgia

EXTENSION:

E. H. LOVELAND, *Chairman*  
University of Vermont  
G. HEEBINK  
University of West Virginia  
C. W. REAVES  
University of Florida  
F. W. FITCH  
University of Georgia

MANUFACTURING:

P. R. ELLIKER, *Chairman*  
Oregon State College  
E. M. BARKER  
University of Minnesota  
D. V. JOSEPHSON  
Ohio State University

PRODUCTION:

G. H. WISE, *Chairman*  
Iowa State College  
L. A. MOORE  
Bureau of Dairy Industry  
D. M. SEATH  
University of Kentucky

REGISTRATION

SOULE HALL  
UNIVERSITY OF GEORGIA

Meetings will be held in the buildings on the campus of the College of Agriculture of the University of Georgia. Headquarters will be in Soule Hall.

The opening session will be in Hardman Hall. All sectional meetings will be in Dawson Hall. Rooms there will be assigned based on the needs of the sections. The general business session on Wednesday afternoon will be in the Auditorium, Dawson Hall.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and 2" x 2" slides. Projectors for 16 mm. movies will be available by ar-

rangement. Please advise respective section chairmen by mail of the type of projection equipment, if any, which will be needed for the presentation of your paper.

#### COMMITTEE MEETINGS

Those wishing rooms for Extension, Production, and Manufacturing Section Committee meetings should write or contact Jennings B. Frye, Jr., Dairy Department, University of Georgia.

#### SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular sessions will please contact Jennings B. Frye, Jr., of the Dairy Department, University of Georgia. Provision can be made for a limited number of breakfasts, luncheons, or dinners for special groups.

#### EXTENSION EXHIBITS

Extension Dairymen desiring space for exhibition of material will please contact Frank W. Fitch, Extension Dairyman, University of Georgia. The exhibits will be in the Auditorium, Dawson Hall.

#### PROGRAM OF ENTERTAINMENT

(Principally for the Ladies)

##### *Monday, June 14*

- 10:00 A.M. TOUR—Antebellum Homes of Athens  
 3:00 P.M. TEA—Founders Memorial Gardens  
                   Compliments of Landscape Architecture Department  
                   and Georgia Feed Manufacturers Association  
 8:00 P.M.\* ENTERTAINMENT—Physical Education Building  
                   Early American Dances—The Atlanta Promenade Club  
                   Negro Spirituals  
                   Group Singing

##### *Tuesday, June 15*

- MORNING FREE. Convenient busses to shopping district  
 12:30 P.M. LUNCHEON—Athens Country Club  
                   Compliments of Kraft Foods Company  
 3:30 P.M. ART EXHIBIT AND DEMONSTRATION—Fine Arts Department  
 8:00 P.M.\* RECEPTION AND DANCE—Memorial Hall  
                   (Formal for Ladies)

##### *Wednesday, June 16*

- 3:00 P.M. BRIDGE—Physical Education Building  
 7:00 P.M.\* BARBECUE—Amphitheatre



8:30 P.M.\* INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS—  
Amphitheatre

\* These features open to all in attendance. Other events open to ladies only. Admission to all events by badge only.

GENERAL PROGRAM

*Monday, June 14*

Eastern Standard Time

9:30-12:00 OPENING SESSIONS, *Hardman Hall*

H. B. HENDERSON, *Dairy Department, University of Georgia,*  
*presiding*

Invocation

DR. PAUL C. HOWLE, *Pastor, First Christian Church,*  
*Athens*

Introduction of Officers and Guests

Address of Welcome

H. W. CALDWELL, *President, University of Georgia*

Presidential Address

PAUL H. TRACY, *President, American Dairy Science Association*

Intermission

Music

Guest Speaker

PAUL W. CHAPMAN, *Dean, College of Agriculture, University of Georgia*

Announcements

1:30- 4:30 SECTIONAL MEETINGS

Production Section A

Genetics and Endocrine Investigations  
*Dawson Hall*

Production Section B

Type, Vitamins, Metabolism, Techniques  
*Dawson Hall*

Manufacturing Section

Dry Milk, Condensed Milk, Ice Cream, Cream  
*Dawson Hall*

Extension Section

Records and Interpretations  
*Dawson Hall*

4:30- 5:30 COMMITTEE MEETINGS

8:00 ENTERTAINMENT, *Physical Education Building*  
Early American Dances—Atlanta Promenade Club  
Negro Spirituals  
Group Singing

*Tuesday, June 15*

9:00-12:00 SECTIONAL MEETINGS

**Production Section A**

Calf Problems  
*Dawson Hall*

**Production Section B**

Artificial Breeding  
*Dawson Hall*

**Manufacturing Section**

Pasteurization, Microbiology, Cheese  
*Dawson Hall*

**Extension Section**

Teaching Methods and Exhibits  
*Dawson Hall*

1:30- 4:00 SECTIONAL MEETINGS

**Joint Meeting of Production and Extension Sections**  
*Dawson Hall*

**Manufacturing Section**

Cheese  
*Dawson Hall*

4:00- 5:30 PRODUCTION AND EXTENSION SECTIONS

**Committee Reports**  
**MANUFACTURING SECTION**  
**Business Meeting**

8:00 RECEPTION AND DANCE, *Memorial Hall*  
(Formal for Ladies)

*Wednesday, June 16*

9:00-11:00 SECTIONAL MEETINGS

**Production Section A**

Parturient Changes in Blood and in Mammary Secretions  
*Dawson Hall*

**Production Section B**

Forages, Hay

*Dawson Hall***Manufacturing Section A**

Chemistry

*Dawson Hall***Manufacturing Section B**

Homogenized Milk, Sanitation, Microbiology

*Dawson Hall***Extension Section**

4-H Clubs and Testing Rules

*Dawson Hall*

11:00-12:00 BUSINESS MEETINGS

Production Section, *Dawson Hall*Manufacturing Section, *Dawson Hall*Extension Section, *Dawson Hall*1:00 GROUP PICTURE, *Amphitheatre*

1:30- 3:00 SECTIONAL MEETINGS

**Production Section A**

Feeding and Management

*Dawson Hall***Production Section B**

Forages, Pastures

*Dawson Hall***Manufacturing Section**

Dairy Sanitation Symposium

*Dawson Hall*3:00- 5:00 GENERAL BUSINESS SESSION, *Auditorium, Dawson Hall*7:00 BARBECUE, *Amphitheatre*

INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS

**PROGRAM OF MANUFACTURING SECTION***Monday, June 14*

Afternoon Session

1:30- 4:30 DRY MILK, CONDENSED MILK, ICE CREAM,  
CREAM. D. V. JOSEPHSON, *Chairman*.M1 The Effect of the Addition of Ascorbic Acid to Milk  
on the Keeping Quality of Its Dried Product. GEORGE  
R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy  
Industry, U.S.D.A.*



- M2 The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*
- M3 The Effect of Heat Treatment on the Reducing Systems of Milk. S. T. COULTER, HERBERT HARLAND, AND ROBERT JENNESS, *University of Minnesota.*
- M3-a The Heat Treatment of Milk Necessary to Prevent Lipolytic Activity in Its Dried Product (A Preliminary Report). GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*
- M4 The Isolation of Compounds Responsible for the Stale Flavor Developed in Dried Whole Milk. I. The Distribution of Stale Flavor between the Fractions of Reconstituted Stale Whole Milk Powder. R. M. WHITNEY AND P. H. TRACY, *University of Illinois.*
- M5 A Solubility Method for the Determination of Alpha and Beta Lactose in Dry Products of Milk. R. P. CHOI, C. W. TATTER, AND B. W. FAIRBANKS, *American Dry Milk Institute, Inc., Chicago, Illinois.*
- M6 The Viscosity and Heat Stability of Milks Subjected to High Temperature Processing. B. H. WEBB AND C. F. HUFNAGEL, *Bureau of Dairy Industry, U.S.D.A.*

## FIFTEEN MINUTE RECESS.

- M7 The Microbiological Keeping Quality of Bulk Condensed Milk. A. M. PEARSON AND F. E. NELSON, *Iowa State College.*
- M8 The Use of Sweetened Condensed Whole Milk in the Manufacture of Caramels. J. J. SHEURING AND P. H. TRACY, *University of Illinois.*
- M9 Influence of the Mineral Content of Water on the Properties of Ice Cream Mixes. ROBERT A. HIBBS AND W. A. KRIENKE, *University of Florida.*
- M10 Observations on the Effects of Various Stabilizing and Emulsifying Materials on the Properties of Ice Cream. W. S. ARBUCKLE, R. B. REDFERN, AND L. F. BLANTON, *North Carolina State College.*
- M11 The Effect of a Mannitol of Beef Fat on the Whipping Qualities, Body and Texture of Ice Cream. RALPH NADEN, J. J. SHEURING AND P. H. TRACY, *University of Illinois.*

- M12 A Study of the Fat Emulsion in Natural and Artificial Creams. W. E. SNYDER, *University of Georgia*, AND H. H. SOMMER, *University of Wisconsin*.

4:30- 5:30 COMMITTEE MEETINGS.

*Tuesday, June 15*

Morning Session

9:00-12:00 PASTEURIZATION, MICROBIOLOGY, CHEESE.

E. M. BARKER, *Chairman*.

- M13 Preservation of Milk for the Phosphatase Test. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*
- M14 Differentiation of Microbial Phosphatases from Milk Phosphatase. RALPH P. TITSTLER, OSCAR S. SAGER, AND GEORGE P. SANDERS, *Bureau of Dairy Industry, U.S.D.A.*
- M15. A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. JOHN HETRICK, *Dean Milk Co., Rockford, Illinois*, AND P. H. TRACY, *University of Illinois*.
- M16 Isolation of Heat-induced Flavor Compounds from Milk. STUART PATTON AND DONALD V. JOSEPHSON, *Ohio State University*.
- M17 Some Observations on the Efficiency of High-temperature Short-time Pasteurization of Chocolate Milk. MARVIN L. SPECK AND CHARLES D. COLVARD, *North Carolina State College*.
- M18 Use of the Direct Microscopic Method for Pasteurized Dairy Products. M. J. PRUCHA AND VIRGINIA FRAZEE, *University of Illinois*.
- M19 Bacteriophage Production by Cultures of *Streptococcus lactis*. F. J. BABEL, *Purdue University*.
- M20 Electron Microscope Studies of Bacteriophages Active against *Streptococcus lactis*. C. E. PARMELEE, P. H. CARR, AND F. E. NELSON, *Iowa State College*.
- M21 Some Factors Affecting the Rate of Acid Production by Cheese Cultures. H. C. OLSON AND FRANCIS D. COHENOUR, *Oklahoma A. and M. College*.
- M22 Methods of Controlling the pH of Fermenting Dairy Products and the Effects of pH Control. WAYNE I. TRETSVEN, *Chicago, Illinois*.

- M23 Chemical Changes Occurring in Limburger Cheese during Accelerated Ripening. W. K. STONE AND S. L. TUCKEY, *University of Illinois*.
- M24 A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-frequency Heat. F. V. KOSIKOWSKY, B. L. HERRINGTON, AND A. C. DAHLBERG, *Cornell University*.
- M25 Increasing Efficiency and Reducing Costs in the Manufacture of Cheddar Cheese. D. M. IRVINE AND WALTER V. PRICE, *University of Wisconsin*.

Tuesday, June 15

Afternoon Session

1:30- 4:00 **CHEESE.** P. R. ELLIKER, *Chairman*.

- M26 The Use of Nonfat Dry Milk Solids in the Manufacture of Cheddar Cheese from High Fat Content Milk. G. H. WILSTER AND C. E. JOHNSON, *Oregon State College*.
- M27 The Problem of Sampling Cheddar Cheese for Analysis. WILLIAM C. WINDER AND WALTER V. PRICE, *University of Wisconsin*.
- M28 The Influence of *Oospora lactis* in Promoting Changes in the Constants of Cheese Fat during Ripening of Cheddar Cheese. S. L. TUCKEY, W. O. NELSON, AND R. V. HUSSONG, *University of Illinois*.
- M29 Studies of Sources of the Typical Flavor in Cheddar Cheese. HAROLD E. CALBERT AND WALTER V. PRICE, *University of Wisconsin*.
- M30 The Influence of Temperature of Ripening on the Concentration of Tyramine in American Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, *Cornell University*.
- M31 Methods for Studying the Ripening of Cheese. H. H. SOMMER AND W. J. HARPER, *University of Wisconsin*.
- M32 The Effect of Added Amino Acids on Flavor Development in Cheddar Cheese. R. J. BAKER AND F. E. NELSON, *Iowa State College*.
- M33 Studies of Amino Acids in Cheddar Cheese during Ripening. W. J. HARPER AND A. M. SWANSON, *University of Wisconsin*.
- M34 The Influence of Various Lactobacilli and Certain Streptococci on the Chemical Changes, Flavor Develop-



ment, and Quality of Cheddar Cheese. R. P. TITSLER, GEORGE P. SANDERS, H. R. LOCHRY, AND O. S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

4:00- 5:00 BUSINESS MEETING.

Wednesday, June 16

Morning Session

9:00-11:00 SECTION A, CHEMISTRY. D. V. JOSEPHSON, *Chairman.*

- M35 Some Physiological Effects of Dietary Lactose. JESSIE ELIZABETH FISCHER AND T. S. SUTTON, *Ohio State University.*
- M36 The Limitations of the Refractometer Readings of Milk Serums in Detecting Watered Milk. L. R. ARRINGTON AND W. L. FOUTS, *University of Florida.*
- M37 The Application of Flame Photometry to Determinations of Calcium, Potassium and Sodium in Milk. W. A. KRIENKE AND NATHAN GAMMON, *University of Florida.*
- M38 A Rapid Method for the Determination of Nitrogen in Milk Products by Direct Nesslerization of the Digested Sample. J. H. HETRICK, *Dean Milk Company, Rockford, Illinois*, AND R. M. WHITNEY, *University of Illinois.*
- M39 Studies on Separation and Fractionation of Casein. E. C. HAGBERG AND A. M. SWANSON, *University of Wisconsin.*
- M40 The Fractionation of Milk Fat by Molecular Distillation. E. L. JACK AND MRS. L. B. OLSEN, *University of California.*
- M41 The Measurement of Free Fatty Acids in Dairy Products. H. A. HOLLENDER AND H. H. SOMMER, *University of Wisconsin.*
- M42 The Determination of Linoleic Acid in Milk Fat. P. S. SCHAFFER AND GEORGE E. HOLM, *Bureau of Dairy Industry, U.S.D.A.*
- M43 Retention of Ascorbic Acid, Changes in Oxidation-reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*
- M44 The Effects of the Treatment of Milk and the Subsequent Storage of Cream and Butter below Freezing

Temperatures upon the Sensitivity of Fat to Oxidative Deterioration as Determined by the Re-emulsification Test. VLADIMIR N. KRUKOVSKY, E. S. GUTHRIE AND FRANK WHITING, *Cornell University*.

- M45 Ascorbic Acid Oxidation in Milk by Preformed  $H_2O_2$ . VLADIMIR N. KRUKOVSKY, *Cornell University*.

Wednesday, June 16

Morning Session

9:00-11:00 SECTION B, HOMOGENIZED MILK, SANITATION, MICROBIOLOGY. E. M. BARKER, *Chairman*.

- M46 Stimulation of the Oxidized Flavors in Homogenized Milk by Light as Governed by the Vitamin C Content of the Milk. E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY, *Cornell University*.
- M47 Studies on Seepage from Bottles of Homogenized Milk. E. O. HERREID, *University of Illinois*.
- M48 The Leucocyte Count of the Complete Milking of Normal Animals for Complete Lactation Periods. E. O. ANDERSON, *University of Connecticut*.
- M49 Effect of Some Water Constituents on Quarternaries. W. S. MUELLER AND D. B. SEELEY, *University of Massachusetts*.
- M50 Germicidal Effectiveness of Certain Hypochlorides and Quaternary Ammonium Compounds under Simulated Plant Conditions. P. R. ELLIKER, *Oregon State College*, AND K. R. SPURGEON, *Purdue University*.
- M51 Sanitizing Milk Cans in Mechanical Can Washers. G. W. REINBOLD, S. L. TUCKEY, R. V. HUSSONG, AND P. H. TRACY, *University of Illinois*.
- M52 Some Factors Involved in Developing a Sediment Test for One-pint Samples of Cream Taken Off the Bottom of the Container. RAYMOND W. MYKLEBY AND BEN M. ZAKARIASEN, *Land-O-Lakes Creameries, Inc., Minneapolis, Minnesota*.
- M53 A Skunk-like Odor of Bacterial Origin in Farm-separated Cream. T. J. CLAYDON, *Kansas State College*.
- M54 Coliform Bacteria in Butter. R. N. SINGH AND F. E. NELSON, *Iowa State College*.

ASSOCIATION ANNOUNCEMENTS

399

- M55 The Effect of *Streptococcus lactis* and Coliform Organisms on Soluble Nitrogen in Milk. E. B. COLLINS AND F. E. NELSON, *Iowa State College*.

11:00-12:00 BUSINESS MEETING.

Wednesday, June 16

Afternoon Session

- 1:30- 3:00 SYMPOSIUM ON ASPECTS OF SANITATION IN THE DAIRY INDUSTRY. P. R. ELLIKER, *Chairman*. K. G. WECKEL, *Leader*.

Chemical and Physical Aspects of Cleaning Dairy Equipment. H. G. HARDING AND H. A. TREBLER, *National Dairy Research Laboratories, Inc., Baltimore, Maryland*.

Aspects of Quarternary Compounds. LUTHER BLACK, *U. S. Public Health Service, Cincinnati, Ohio*.

Bacteriophage and Its Relation to Sanitary Practices. F. J. BABEL, *Purdue University*.

- 3:00- 5:00 GENERAL BUSINESS SESSION, *Auditorium, Dawson Hall*.

- 7:00 BARBECUE, *Amphitheatre*.

PROGRAM OF PRODUCTION SECTION

Monday, June 14

Afternoon Session

- 1:30- 4:30 SECTION A, GENETICS AND ENDOCRINE INVESTIGATIONS. G. H. WISE, *Chairman*.

P1 The Relative Merits of a Cow's Own Record and Her Progeny Test for Predicting the Butterfat Production of Her Future Daughters. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University*.

P2 Preliminary Results from the Crossing of Two Inbred Lines of Holsteins on Growth and Milk Production. N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, *University of California*.

P3 Genetic Variation in the Levels of Blood Plasma Carotene and Vitamin A in Dairy Cattle. R. E. MATHER, *New Jersey Agricultural Experiment Station*.

P4 Measurement of the Rate of Endocrine Gland Secretion as a Tool in the Genetic Selection of Dairy Cattle. C. W. TURNER, *University of Missouri*.



- P5 Thyroid Secretion Rate and Its Relation to Various Physiological Processes. VICTOR HURST, *University of Missouri*.
- P6 The Effect of Low Levels of Thyroprotein Feeding upon Milk and Milk Fat Production, Body Weight, Body Temperature, Heart Rate and Respiration Rate of Dairy Cows. R. G. SWANSON AND C. B. KNOTT, *Pennsylvania State College*.
- P7 Effects of Feeding Thyroprotein to Milking Cows in Summer. K. E. GARDNER AND T. W. MILLEN, *University of Illinois*.
- P8 Effects of Feeding Thyroprotein during Successive Lactations. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
- P9 Factors Controlling the Extent of Duct Growth in Mammary Glands. I. The Influence of an Estrogen in a Hereford Heifer. RALPH P. REECE, *New Jersey Agricultural Experiment Station*.
- P10 The Value of Oxytocin for Reducing Fluctuations in Milk and Fat Yield during Experimental Periods. H. P. ADAMS AND N. N. ALLEN, *University of Wisconsin*.
- P11 The Role of Certain Hormones in Spermatogenesis. J. D. SAMPATH KUMARAN, *University of Missouri*.

Monday, June 14

Afternoon Session

1:30- 4:30 SECTION B, TYPE, VITAMINS, METABOLISM, TECHNIQUES. L. A. MOORE, *Chairman*.

- P12 The Relationship between Type Rating of Ayrshire Females as Young Heifers and as Cows. GEORGE HYATT, JR., AND W. J. TYLER, *West Virginia University*.
- P13 The Effect of Certain Vitamins and Minerals on Blood Carotene Values of Dairy Animals. DWIGHT ESPE, *North Dakota Agricultural College*.
- P14 Effect of Certain Soybean Products on the Concentrations of Carotene and Vitamin A in the Milk and in the Blood Plasma of Dairy Cows. R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN, *Iowa State College*.
- P15 Further Studies on the Relationship between the Feeding of Soybeans and the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE, J. C. SHAW, AND B. C.

- HATZIOLOS, *University of Maryland*, AND L. A. MOORE AND J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*
- P16 The Influence of Tocopherols on the Fat Content of Milk. F. WHITING AND J. K. LOOSLI, *Cornell University*.
- P17 Covitamin Studies of Milk Fats from Four Breeds of Dairy Cattle. V. N. KRUKOVSKY AND F. WHITING, *Cornell University*.
- P18 Heat Production and Cardiorespiratory Activities during Gestation and Lactation in Jersey Cattle. S. BRODY, D. M. WORSTELL, H. H. KIBLER, AND A. C. RAGSDALE, *University of Missouri*.
- P19 A Biochemical and Histo-pathological Study of Ketosis in Dairy Cattle. J. C. SHAW, B. C. HATZIOLOS, AND V. P. SAARINEN, *University of Maryland*.
- P20 A Study of Sampling at Various Stages of Milking in Determining the Bacterial Flora of the Udders of Dairy Cows. E. M. KESLER, C. B. KNOTT, AND J. T. REID, *Pennsylvania State College*.
- P21 A Permanent and Convenient Rumen Fistula for Dairy Cows. G. E. STODDARD AND N. N. ALLEN, *University of Wisconsin*.
- P21-a Studies Bearing on the Bloat Problem. H. H. COLE AND MAX KLEISER, *University of California*.

4:30- 5:30 COMMITTEE MEETINGS.

*Tuesday, June 15*

Morning Session

- 9:00-12:00 SECTION A, CALF PROBLEMS. G. H. WISE, *Chairman*.
- P22 Calf Losses in a Self-contained Herd over a Period of 17 Years. R. E. JOHNSON, E. L. JUNGHER, AND W. N. PLASTRIDGE, *University of Connecticut*.
- P23 The Effect of Prepartum Vitamin A Supplementation on the Newborn Calf. A. A. SPIELMAN, H. D. EATON, J. K. LOOSLI, AND K. L. TURK, *Cornell University*.
- P24 The Utilization of Fetal Liver Stores of Vitamin A by the Newborn Calf. A. A. SPIELMAN, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut*

- P25 Effect of the Method of Administration of Carotene and of Vitamin A upon the Rate at Which They Are Absorbed from the Alimentary Tract of Dairy Calves. N. L. JACOBSON, G. H. WISE, AND R. S. ALLEN, *Iowa State College*.
- P26 Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced in Calves by Ration Changes. W. C. JACOBSON AND J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*
- P27 Influence of the Ration on Some Blood Vitamin Constituents of the Young Dairy Calf. JOHN W. HIBBS AND W. D. POUNDEN, *Ohio Agricultural Experiment Station*.
- P28 Influence of the Ration on the Digestive Tract Microorganisms of the Young Dairy Calf. W. D. POUNDEN AND JOHN W. HIBBS, *Ohio Agricultural Experiment Station*.
- P29 Relation of Aerobic Bacterial Flora to Consistency of Feces. M. D. VAN PELT, R. E. JOHNSON, AND W. N. PLASTRIDGE, *University of Connecticut*.
- P30 Raising Dairy Calves without Colostrum. J. T. MILES, S. A. HINTON, AND HOMER PATRICK, *University of Tennessee*.
- P31 A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN, AND F. THORP, *Michigan State College*.
- P32 Effect of Tryptophan in the Diet on the Excretion of Niacin and Its Metabolic Products in Dairy Calves. G. C. ESH AND T. S. SUTTON, *Ohio State University*.
- P33 Performance of Calves on a Photolysed Milk Diet. R. G. WARNER AND T. S. SUTTON, *Ohio State University*.
- P34 Anemia in Young Calves and Its Alleviation by Iron. W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

PAUL H. PHILLIPS, *Discussion Leader*.

9:00-12:00 SECTION B, ARTIFICIAL BREEDING. L. A. MOORE, *Chairman*.

- P35 A Method of Evaluating Bull Semen. TOM LUDWICK, D. OLDS, AND M. CARPENTER, *University of Kentucky*.



- P36 Vital Staining of Bovine Spermatozoa with an Eosin-aniline Blue Staining Mixture. H. E. SHAFFER AND J. O. ALMQUIST, *Pennsylvania State College*.
- P37 Turbidometric Assay of Hyaluronidase in Bull Semen. JOHN P. MIXNER AND JAMES E. JOHNSTON, *New Jersey Agricultural Experiment Station*.
- P38 Hyaluronidase and Bull Semen. J. E. JOHNSON, E. J. STONE, AND J. P. MIXNER, *New Jersey Agricultural Experiment Station*.
- P39 Effect of Testis Biopsy on Semen Characteristics. J. F. SYKES, W. J. SWEETMAN, AND P. C. UNDERWOOD, *Bureau of Dairy Industry, U.S.D.A.*
- P40 Spermatozoa Behavior in Bovine Cervical Mucus at Varying Stages of Estrus. H. A. HERMAN AND OTIS H. HORTON, *University of Missouri*.
- P41 Varying the Proportion of Egg Yolk in Diluters for Bull Semen. ERIC W. SWANSON, *University of Tennessee*.
- P42 A Study of the Types of Bacteria in Bovine Semen and Their Effect upon Motility. J. E. EDMONDSON, K. L. TALLMAN, AND H. A. HERMAN, *University of Missouri*.
- P43 Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. J. O. ALMQUIST, *Pennsylvania State College*.
- P44 Breeding Results with Bovine Semen Treated with Varying Amounts of Thyroxine. A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska*.
- P45 Measuring Breeding Efficiency by Pregnancy Examinations and by Non-returns. G. R. BARRETT, L. E. CASSIDA, AND C. A. LLOYD, *University of Wisconsin*.
- P46 Order Number of Insemination and Conception Rate. G. R. BARRETT, C. A. LLOYD, AND R. A. CARPENTER, *University of Wisconsin*.
- G. W. SALISBURY, *Discussion Leader*.

*Tuesday, June 15*

Afternoon Session

- 1:30- 4:30 JOINT MEETING WITH EXTENSION SECTION.  
E. H. LOVELAND AND G. H. WISE, *Co-Chairmen*.  
Symposium—Reproductive Problems in Dairy Cattle. L. A. MOORE, *Leader*.

1. Infectious Disease as a Cause of Infertility. D. E. BARTLETT, *Bureau of Animal Industry, U.S.D.A.*
2. Functional Causes of Infertility and Methods of Treatment.
  - a. Hormone Disturbances } S. A. ASDELL, *Cornell University.*
  - b. Nutrition Disturbances {
  - c. Inheritance. L. O. GILMORE, *University of Minnesota.*
3. Possible Modes of Approach to a Study of Infertility. J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*
4. Activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association. P. H. PHILLIPS, *University of Wisconsin.*

#### 4:30- 5:30 COMMITTEE REPORTS.

*Dairy Cattle Health Committee.* L. A. MOORE, *Chairman.*

*Dairy Cattle Breeding Committee.* E. J. PERRY, *Chairman.*

*Breeds Relations Committee.* H. A. HERMAN, *Chairman.*

1. Program of Purebred Dairy Cattle Association. G. A. BOWLING, *Sec.-Treas.*

Wednesday, June 16

Morning Session

#### 9:00-11:00 SECTION A, PARTURIENT CHANGES IN BLOOD AND IN MAMMARY SECRETIONS. G. H. WISE, *Chairman.*

- P47 The Effect of Udder Inflation of Cows with Parturient Paresis on Blood Calcium, Magnesium and Inorganic Phosphorus. YEAL R. SMITH AND R. P. NEIDERMEIER, *University of Wisconsin.*
- P48 A Study of Citric Acid Levels in the Blood and Urine of Cows at Time of Parturition. T. H. BLOSSER, YEAL R. SMITH, AND H. A. LARDY, *University of Wisconsin.*
- P49 The Effect of Prepartum Milking on Some Blood Constituents of the Cow. R. E. JOHNSON, H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut.*
- P50 A Study of Some Blood Constituents of Cows not Milked Following Parturition. R. P. NEIDERMEIER AND YEAL R. SMITH, *University of Wisconsin.*

- P51 The Effect of Preparturient Milking on the Composition of Colostrum. A. H. VAN LANDINGHAM, C. E. WEAKLEY, R. A. ACKERMAN, AND GEORGE HYATT, JR., *West Virginia University*.
- P52 The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut*.
- P53 The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, R. E. JOHNSON, AND R. J. SLATE, *University of Connecticut*.
- P54 The Effect of the Form of Vitamin A and of Tocopherol Supplements of the Ration on the Concentration of Vitamin A and Carotenoids of Colostrum and Early Milk. D. B. PARRISH, GEORGE H. WISE, AND J. S. HUGHES, *Kansas State College*.
- T. S. SUTTON, *Discussion Leader*.

9:00-11:00 SECTION B, FORAGES, HAY. L. A. MOORE, *Chairman*.

- P55 Comparison of Barn-cured and Field-cured Alfalfa Hay. GILBERT H. ROLLINS AND PAUL M. REAVES, *Virginia Polytechnic Institute*.
- P56 Studies on Mow Curing of Baled Hay. W. A. KING, J. W. WILBUR, S. M. HAUGE, AND A. W. COOPEL, *Purdue University*.
- P57 Stack Finishing of Baled Hay with and without Heat. K. A. KENDALL, W. B. NEVENS, AND J. H. RAMSER, *University of Illinois*.
- P58 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and a Poor Quality Field-cured Hay. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, C. G. MELIN, W. J. SWEETMAN, W. H. HOSTERMAN, AND H. M. TYSDAL, *Bureau of Dairy Industry; Bureau of Plant Industry, Soils and Agricultural Engineering; and Production and Marketing Administration*.
- P59 Vitamin D Content of Forages as Affected by Various Curing Procedures. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
- P60 Comparison of Early-cut and Late-cut Lespedeza Hay for Milk Production. C. E. WYLIE, J. A. EWING,



ERIC W. SWANSON, AND J. N. MADDUX, *University of Tennessee.*

P61 The Influence of Various Hays on the Production, Vitamin Content, and Flavor of Milk. J. K. LOOSLI, V. N. KRUKOVSKY, AND G. P. LOFGREEN, *Cornell University.*

P62 Comparison of Digestion Coefficients of Sun-cured and Mow-cured Hays from the Same Field. O. M. CAMBURN, *University of Vermont.*

11:00-12:00 BUSINESS MEETING.

Wednesday, June 16

Afternoon Session

1:30- 3:00 SECTION A, FEEDING AND MANAGEMENT. G. H. WISE, *Chairman.*

P63 Lactating Factors for Dairy Cows in Dried Grapefruit Peel. R. N. DAVIS AND A. R. KEMMERER, *University of Arizona.*

P65 The Growth of Dairy Heifers Reared on Maximum Roughage with Varying Amounts of Grain. O. T. STALLCUP, H. A. HERMAN, AND A. C. RAGSDALE, *University of Missouri.*

P66 Wintering Dairy Heifers on Legume Hay. S. A. HINTON, J. T. MILES, AND C. E. WYLIE, *University of Tennessee.*

P67 Observations on Calves Dehorned with Antimony Trichloride-salicylic Acid-collodion Preparation. G. E. STODDARD, *University of Wisconsin.*

P68 Comparison between Various Methods of Cooling Dairy Cows in Summer. D. M. SEATH AND G. D. MILLER, *Louisiana Agricultural Experiment Station.*

P69 Relationship of Management to the Let-down of Milk. C. E. KNOOP, *Ohio Agricultural Experiment Station.*

P70 The Effect of Time of Milking after Milk Excretion on Total Milk Production. G. M. WARD AND VEARL R. SMITH, *University of Wisconsin.*

1:30- 3:00 SECTION B, FORAGES, PASTURES. L. A. MOORE, *Chairman*.

P71 Silage or Winter Pasture for Dairy Cattle. C. E. WYLIE, S. A. HINTON, AND L. R. NEEL, *University of Tennessee*.

P72 Sweet Sudan as a Forage Crop for Dairy Cattle. K. A. KENDALL AND W. B. NEVENS, *University of Illinois*.

P73 Pastures in Relation to Dairy Development in the South. R. H. LUSH, *University of Tennessee*.

P74 Irrigated Pastures for Dairy Cows. JOHN EWING, NELSON MADDUX, C. E. WYLIE, AND R. H. LUSH, *University of Tennessee*.

P75 Increasing the Production of Permanent Pastures through Renovation. J. B. SHEPHERD, R. E. WAGNER, R. E. HODGSON, W. J. SWEETMAN, AND C. G. MELIN, *Bureau of Dairy Industry and Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S.D.A.*

P76 Effect of Intermittent and Limited Winter Grazing of Rye Pasture on the Carotene and Vitamin A Content of Cows' Milk. R. G. WASHBURN AND C. F. MONROE, *Ohio Agricultural Experiment Station*.

3:00- 5:00 GENERAL BUSINESS SESSION. *Auditorium, Dawson Hall*.

7.00 BARBECUE, *Amphitheatre*.

#### PROGRAM OF EXTENSION SECTION

*Monday, June 14*

Afternoon Session

1:30- 4:30 RECORDS AND INTERPRETATION. E. H. LOVE-  
LAND, *Chairman*.

Opening Business Session.

E1 Report of Dairy Records Committee. CHARLES GEAR-  
HART, *Pennsylvania State College*.

E2 Seven Years of Central Laboratory Testing. J. E.  
STOLLARD, *University of Wisconsin*.

Discussion.

*Tuesday, June 15*

Morning Session

9:00-12:00 **TEACHING METHODS AND EXHIBITS.**—G. HEEBINK, *Chairman.*

E3 Report of Committee on Teaching Methods. I. L. PARKIN, *Pennsylvania State College.*

E4 Interdepartmental Cooperation on Dairy Extension. EVERT WALLENFELDT, GEORGE WERNER, AND CARL NEITZKE, *University of Wisconsin.*

Explanation and Discussion of Exhibits, *Auditorium, Dawson Hall*

Afternoon Session

1:30- 4:00 **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS.** E. H. LOVELAND AND G. H. WISE, *Co-Chairmen.*

(See Production Section Program)

*Wednesday, June 16*

Morning Session

9:00-11:00 **4-H CLUB AND TESTING RULES.** E. H. LOVELAND, *Chairman.*

E5 Systems Used in Obtaining 4-H Club Calves. RALPH PORTERFIELD, *University of Maryland.*

E6 National and Regional 4-H Dairy Contests. M. J. REGAN, *University of Missouri.*

E7 Adoption of Practices as the Result of 4-H Dairy Work. J. C. NAGEOTTE, *Pennsylvania State College.*

E8 Suggested Revision of D.H.I.A. Rules and Regulations. CHARLES GEARHART, *Pennsylvania State College.*

Discussion.

Afternoon Session

3:00- 5:00 **GENERAL BUSINESS SESSION,** *Auditorium, Dawson Hall.*

7:00 **BARBECUE,** *Amphitheatre.*



# JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

JUNE, 1948

NUMBER 6

## THE SPECTROPHOTOMETRIC DETERMINATION OF THE COLOR OF MILK

VICTOR NELSON

*Central Research Department, Food Machinery Corporation, San Jose, California*

In the commercial manufacture of evaporated milk, considerable effort is given to the production of milk of uniform quality throughout the year. While ideas on quality vary, flavor, color and viscosity generally are regarded as the chief factors in quality rating.

In order to have a record of quality ratings, some method of measurement must be used which can be related to an accepted standard. Viscosity can be determined easily, but color and flavor have been difficult to rate, since no convenient or wholly satisfactory standards have been available. In most laboratories color and flavor remain a matter of the personal judgment of the inspector. However, color can be referred to known standards. The purpose of this paper is to report on the spectrophotometer as a means of evaluating the color of evaporated milk and related products.

### METHODS AND APPARATUS

Some years ago Webb and Holm (4) and more recently Bell and Webb (1) measured the color produced in the processing of evaporated milk by means of the Munsell system of disc colorimetry. This system is relatively convenient, inexpensive and fairly accurate in its specifications of color. However, its lack of high sensitivity excludes it from the measurement of the minute changes in color which accompany variations in the heat processing of milk, especially those changes occurring at the lower temperatures, *e.g.*, at 220° F.

In recent years several spectrophotometers of relatively low cost have been introduced, and among these the Beckman provides a reflectance attachment for measuring the color of opaque solids. This attachment is so designed that it easily can be adapted for the measurement of opaque liquids such as milk.

Since no containers for liquids were included in the equipment, it was necessary to construct them in the laboratory. The containers were constructed from tin plate, were circular in shape, 0.5 inch in depth and 1.125

Received for publication December 6, 1947.

inches in diameter. The reference standard ordinarily used is a magnesia block, but some difficulty was experienced in obtaining a block of uniformly high reflectance. Hence, for the data herein reported, the standard used was one of the sample cups filled with reagent grade magnesium carbonate. For simplicity, this standard was considered as having a reflectance of 100 per cent. A weighed amount of milk was used in order to insure a constant depth of milk in the cup. Since the surface of the reference magnesia standard and the surface of the liquid should be at the same level for accurate comparison, the surface of the standard was lowered to the level of the milk by the insertion of a metal plate with a 1-inch diameter circular opening and proper thickness between the top of the cup and the retaining plate. Measurements then were made as usual over the wave front of the instrument.

#### EXPERIMENTAL

The experimental part of this work consisted in comparing the reflectance of milk samples before and after processing with the reflectance of the

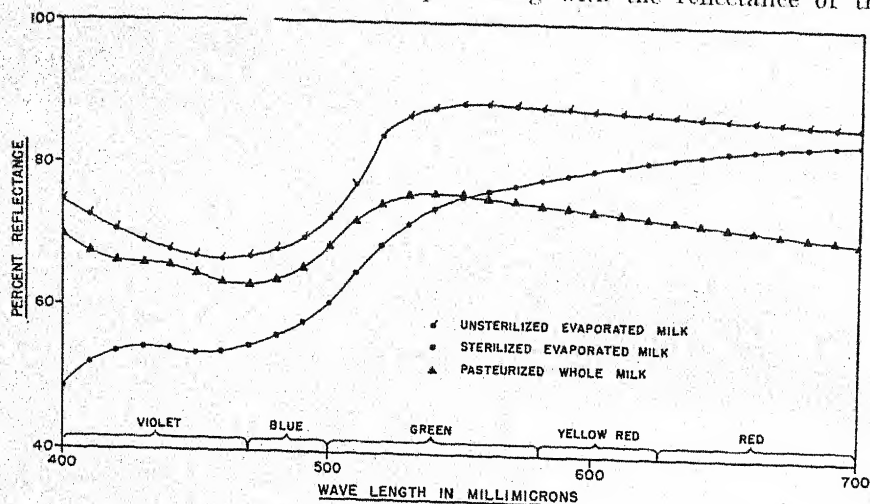


FIG. 1. Variations in reflectance values of pasteurized whole milk, sterilized milk and unsterilized milk between 400-700 mμ.

magnesia standard. These samples consisted of pasteurized milk, unsterilized evaporated milk, sterilized evaporated milk, and six lots of evaporated milk which had received varying preheater and sterilization treatments.

A comparison of the color of pasteurized milk with unsterilized evaporated milk and sterilized evaporated milk is shown by the curves on figure 1. The color difference between the three curves is indicated by their relative positions. The brightness of each color is indicated by the average level of the curve, *e.g.*, the color of unsterilized evaporated milk is the brightest,

with the pasteurized milk and the sterilized milk averaging about the same. An accurate statement regarding hue shift is possible only on the basis of colorimetric data to be calculated from these curves. The saturation of each color is indicated by the relative slope of the curves; *e.g.*, the pasteurized milk curve is the flattest, therefore the least saturated, while the other curves are steeper and therefore more saturated.

It should be noted that the spectrophotometer provides a means for the analysis of the spectral composition of a color sample, while the visual impression is the effect produced on the observer by the combined effect of the spectral composition of the sample, the spectral composition of the illuminant under which the sample is viewed, and the observer's own visual mechanism (which is more receptive to wave lengths in the middle portion of the visible spectrum than those on either end).

With regard to the data plotted on figure 1, it is interesting to note that concentration of milk produces an increase in reflectance in the green, yellow, and red wave lengths but little change in the blue and violet. There

TABLE 1  
*Conversion of curve data into I.C.I. and Munsell notation*

Curve	I.C.I. color notation			Munsell color notation		
	x	y	Y	Hue	Value	Chroma
Pasteurized milk .....	0.3330	0.3470	0.738	7.3Y	8.8	1.7
Unster. evap. milk .....	0.3315	0.3435	0.853	5.8Y	9.3	1.55
Ster. evap. milk .....	0.3435	0.3500	0.738	1.7Y	8.8	2.3

is a possibility that the decrease in the blue-violet region is produced in the forewarming and evaporation processes.

Sterilization of milk produces a marked decrease in reflectance at all wave lengths, especially marked in the violet region. This inequality in reflectance loss is the primary reason for the brown appearance of sterilized evaporated milk, since the result is a relative increase in red and yellow and not an actual increase in these colors.

The conversion of the spectrophotometric data into the I.C.I. (2) and Munsell notation (3) is given in table 1. The Munsell values derived are in good agreement with those obtained by Bell and Webb (1) on evaporated milk. Therefore, it appears that no serious error is introduced by the fluorescence of riboflavin or other compounds.

In the routine grading of freshly sterilized evaporated milk, it is desirable to know the relative color of milk in terms of a simple index number. While this index number cannot represent accurately the true color, it can indicate the direction of shift in hue, brightness and chroma and thus afford to the inspector a quick estimate of the change in color. Since the



heat treatment of milk produces a loss of reflectance, especially marked in the green region, a wave length of 520  $m\mu$  appears particularly suitable for routine work on standard evaporated milk, since small visual changes give large instrumental readings. Furthermore, no special light bulb is needed, since the ordinary light source is relatively strong at this wave length.

The data plotted on figure 2 provide a comparison of the spectral composition of sterilized and unsterilized milk over a greater range than that provided by the data in figure 1. In this second experiment it was desirable to measure the degree of darkening, from a visual point of view, produced in the high temperature pretreatment of evaporated milk and in the subse-

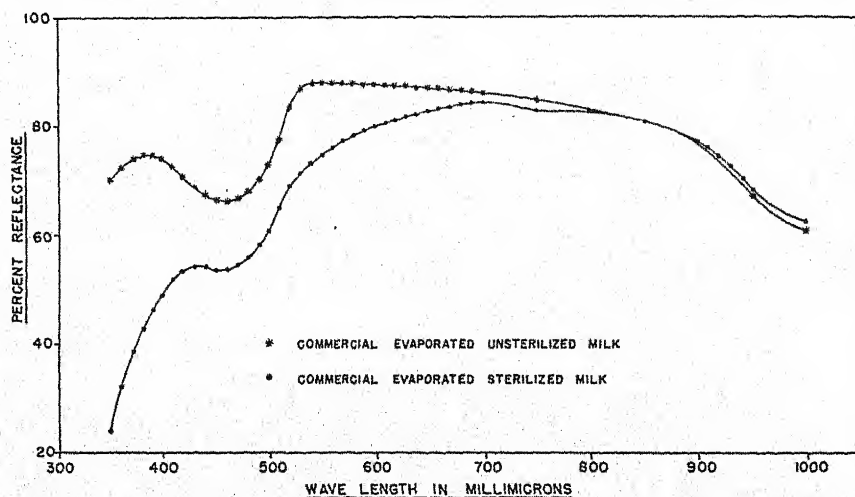


FIG. 2. Variations in reflectance values of sterilized and unsterilized milk between 350-1000  $m\mu$ .

quent sterilization. Since these data are from pilot plant research on high temperature-short time sterilization, a note of explanation is offered.

The data are presented because they represent extreme time-temperature variations in treatment, interesting for the purpose of illustrating the effect on color values. A brief outline of the data follows: The treatment raised the temperature of the milk to the desired temperature in a few seconds. The pretreatment time (table 2) refers to the time the evaporated milk was held at the given temperature in a jacketed holder. The corresponding reflectance value was that taken after rapid cooling of the milk.

The cooled evaporated milk was filled into cans of 14.5-ounce capacity. The filled cans then were closed and treated in an experimental continuous sterilizer as follows: The cans were conveyed through a pre-heating chamber, the temperature of which increased at a uniform rate from 225° F. at the portal of entrance to 235° F. at the portal of exit of the cans. The cans of

TABLE 2  
The effect of pretreatment and sterilization on the reflectance value of milk  
(Sterilization time was 4.7 min.)

Group	Lot no.	Pretreatment temp. (°F.)	Pretreatment time (min.)	% Reflectance 520 mμ	Sterilizer temp. (°F.)	% Reflectance 520 mμ	Viscosity M.U. <sup>a</sup>
I	A	215	0	78.8	261	67.7	92
	B	"	8	78.3	260	68.2	50
	C	"	16	77.5	255	68.2	70
	D	"	24	75.9	254	66.2	85
	E	"	32	74.4	255	67.7	32
II	A	220	0	80.2	262	68.7	47
	B	"	5	80.1	256	66.5	48
	C	"	10	78.4	259	67.7	66
	D	"	15	77.4	255	64.5	80
	E	"	20	73.8	252	65.4	66
III	A	230	0	80.2	261	69.6	41
	B	"	4	79.3	259	66.2	25
	C	"	8	77.6	259	65.4	70
	D	"	12	75.7	254	62.2	125
	E	"	16	77.5	252	62.2	106
IV	A	240	0	76.9	260	68.0	39
	B	"	3	78.0	259	64.8	20
	C	"	6	73.9	259	61.6	68
	D	"	9	73.5	254	58.6	105
	E	"	12	71.2	252	62.2	52
V	A	250	0	77.6	262	69.0	19
	B	"	2	75.7	259	58.4	50
	C	"	3.5	75.4	257	59.8	50
	D	"	5	71.8	254	57.2	15
	E	"	6	71.2	262	60.7	37
VI	A	260	0	75.6	260	67.3	22
	B	"	0.5	74.4	262	58.4	18
	C	"	1.0	75.0	262	59.2	20
	D	"	1.5	66.0	254	51.3	12
	E	"	2.0	67.7	.....	57.3	15

<sup>a</sup> M.U. = (Centipoise + 10)/1.9 (approximate).

milk then were conveyed through a second chamber, where they were subjected to the indicated temperature for 4.7 minutes. After this sterilization treatment the samples were cooled in the usual manner and tested for reflectance loss and viscosity. Viscosity values are in terms of Mojonnier units. There was no "burn-on" or other abnormality which would affect viscosity or color values.

Some discrepancies may be noted in the data. These could well be due to variations in the color of the original milk used and to some unavoidable departures from the temperatures given. In the case of the sterilized product, variations in the rate of cooling affected the color. In any case deviations from the expected color are not large when considered from the standpoint of visual perception, except for some notable exceptions in Groups V and VI. In these latter groups considerable unexplained variation was found in the sterilizing and color characteristics of the various lots of milk.

#### SUMMARY

1. The Beckman spectrophotometer provides basic data for spectral composition of energy reflected from a sample and when it is combined with standard colorimetric data (as the I.C.I. Standard Observer and one of the I.C.I. Standard Illuminants) it provides a good means of estimating the color.

2. A convenient index for routine estimations of the darkening in color of evaporated milk can be determined by noting changes in reflectance of light of 520 m $\mu$  wave length.

The author is indebted to Mr. Paul C. Wilbur, A. E. Pech and Dr. C. R. Stumbo for their valuable suggestions and criticism.

#### REFERENCES

- (1) BELL, R. W., AND WEBB, B. H. The Relationship between High Temperature Forewarming and the Color and Heat Stability of Evaporated Milk of Different Solids Content. *J. Dairy Sci.*, 26: 579-585. 1943.
- (2) HARDY, A. C. Handbook of Colorimetry. The Technology Press, Mass. Inst. of Technol., Cambridge, Mass. 1936.
- (3) NICKERSON, D. Color Measurement and Its Application to the Grading of Agricultural Products. Miscellaneous Pub. 580, U. S. Dept. of Agr. 1946.
- (4) WEBB, B. H., AND HOLM, G. E. Color of Evaporated Milks. *J. Dairy Sci.*, 13: 25-39. 1930.



## THE COLOR OF EVAPORATED MILK WITH RESPECT TO TIME AND TEMPERATURE OF PROCESSING

VICTOR NELSON

*Central Research Department, Food Machinery Corporation, San Jose, California*

It is well known in the evaporated milk industry that the color of evaporated milk can be improved by using a high-temperature-short-time sterilization process. Recently, Tarassuk (3) showed that color in evaporated milk could be reduced a significant amount by reducing the oxygen content of the milk before sterilization. However, there is almost no information available, except that given by Bell and Webb (1), on the rates of color formation at the various sterilization temperatures.

Information on the rate of color development always has been desirable, but until recently no entirely satisfactory method has been available. In this paper the technique of color measurement used by Nelson (2) is applied to the investigation of the rates of color formation during the processing of evaporated milk.

### METHOD AND APPARATUS

The apparatus consisted of a thermostatically controlled oil bath, a preliminary heating oil bath maintained at 175° F., 75 mm. x 10 mm. test tubes, a wire tray for holding the tubes, a cold water bath for cooling the tubes quickly after heating and a Beckman spectrophotometer for reflectance measurements.

Because of the small milk sample used, a small container made from plastic was used instead of the larger container used by Nelson (2). Tests were made to insure comparableness of the two containers.

One and one-half milliliters of commercial unsterilized evaporated milk of 26 per cent total solids content was inserted carefully into the small tubes with the aid of a hypodermic needle. The tubes were sealed over a small pointed flame, the hot tip being drawn into a loop so that it could be suspended on a wire and placed in the wire basket.

The desired number of tubes filled with the evaporated milk was placed in the basket and held in the preliminary oil bath for 3 minutes before immersion in the constant temperature process bath. After immersion in the process bath, tubes were withdrawn at stated intervals, cooled in the water bath, dried, numbered and later analyzed.

### EXPERIMENTAL

The data obtained in this work are represented graphically in figures 1 to 5.

Received for publication December 6, 1947.

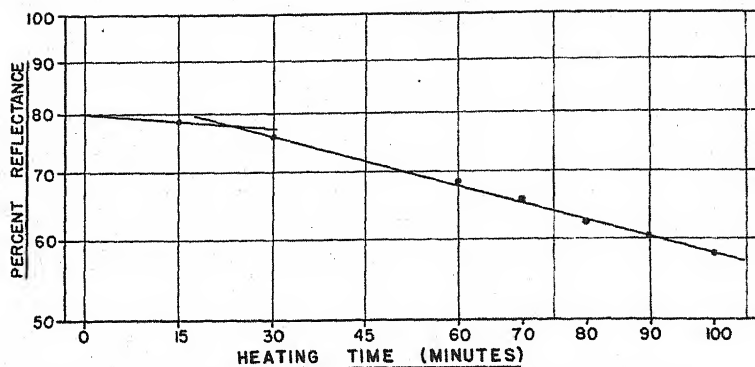


FIG. 1. The relationship between time of heating at 220° F. and reflectance at 520  $m\mu$  wave length.

The preliminary heating of the tubes to 175° F. is not essential but it is convenient, since the time then necessary to arrive within a degree of the desired temperature in the process oil bath is reduced to approximately 3 minutes, as determined by thermocouple measurements and the well known logarithmic nature of the heat penetration curve. Consequently, zero time in this experiment is 3 minutes after immersion in the process oil bath.

The data are plotted on semi-logarithmic paper, since it was found that a straight line was obtained if the logarithm of the reflectance was plotted against time.

Some reflectance loss is noted at zero time at 250° F. (fig. 4). However, all the data are plotted without correction, since the lag in the reflectance loss at the lower temperatures, or the rate of loss, is so low that a measurable reflectance loss cannot be found for several minutes. In any case, the error in zero time does not affect the slope of the curves, although it

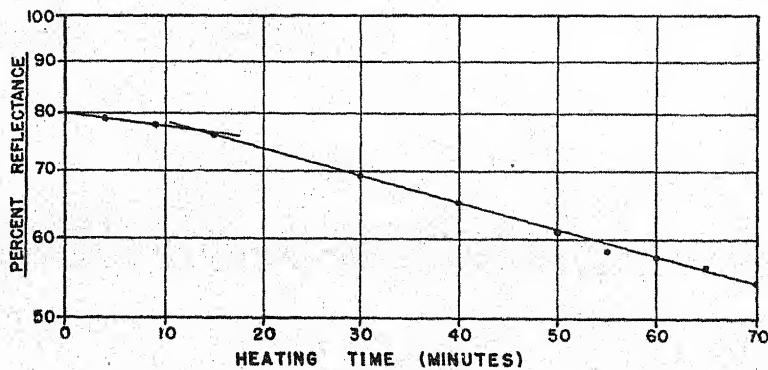


FIG. 2. The relationship between time of heating at 230° F. and reflectance at 520  $m\mu$  wave length.

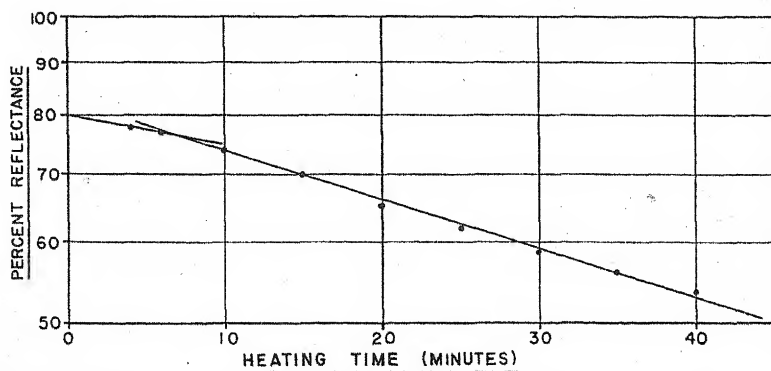


FIG. 3. The relationship between time of heating at 240° F. and reflectance at 520  $m\mu$  wave length.

does affect, to a minor degree, the values of the 250° F. curve. However, if it is desired to correct for this loss it seems not unreasonable to assume that projection of the curve until it crosses the 80 per cent line will give the time—about 30 seconds in this case—which should be added to the time plotted. (The reflectance of the original milk was 79.8 per cent at 520  $m\mu$  wave length.)

Plotting the data on semi-logarithmic paper was found advantageous. The data for the 250° F. curve are represented by a single straight line, while the data for the other curves are represented most conveniently by two straight lines. While the data for the short curves are inconclusive in determining the character of the curves, they are represented as straight lines for convenience and also to indicate the change in slope of the longer curves. In any event, there is a lag in the darkening in color of evaporated milk during processing, a situation also noted by Townley and Gould (4), who found that a visible color change occurred at the time of marked de-

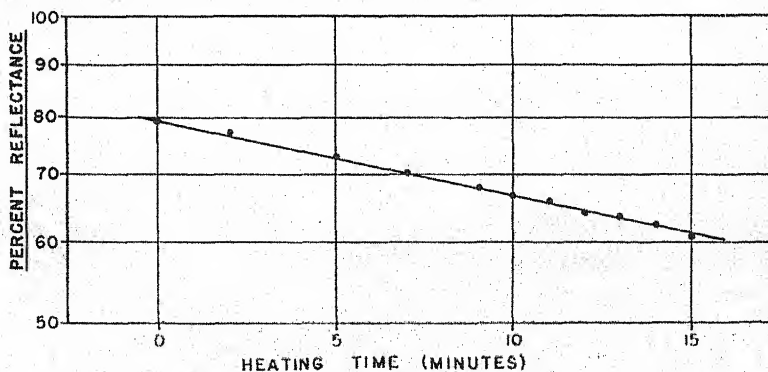


FIG. 4. The relationship between time of heating at 250° F. and reflectance at 520  $m\mu$  wave length.



crease in labile sulfur liberation. Whether this point of decrease marks a decided increase in the oxidation-reduction potential has not been determined, but in view of the effect of oxygen on color, it may be significant.

Curves 1 and 2 on figure 5 were derived from the slopes of the curves marked 220°, 230°, 240° and 250° F. The numerals on Curve 1 indicate the time in minutes at these particular points for which this curve is valid. After this time period, values should be selected from Curve 2.

In connection with this experiment, it should be noted that the ratio of volume of air to milk is greater than in commercial canning. Therefore,

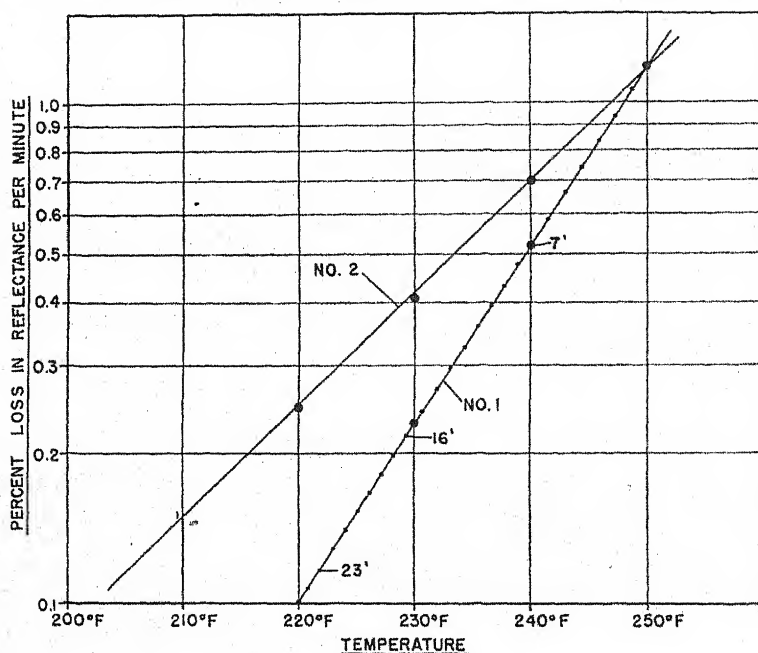


FIG. 5. The relationship between temperature and reflectance loss per minute.

it is quite possible that Curve 1 represents the condition of minimum lag period likely to be encountered in commercial practice. On the other hand, Curve 2 normally will represent the conditions prevailing after the lag phase of sterilization is concluded.

The data obtained in this experiment find application in the color evaluation of sterilization processes. While it is difficult to arrive at absolute values because of the variables introduced by the pretreatment a milk receives, the relative color values of processes can be determined with reasonable accuracy. Given the heat penetration curve of a process, a new curve can be constructed by substituting rate of reflectance loss values for temperature and integrating graphically the curve produced. For example, it will be found that the color produced in a commercial cooker

process of 15 minutes at 243° F. is greater than a comparable process of 6 minutes at 254° F. Not only is the high temperature process short, but the reflectance loss values are relatively low, since a large part of the process occurs in the lag phase of the curve.

#### SUMMARY AND CONCLUSIONS

1. The loss in reflectance at the temperatures studied decreased logarithmically with time after a lag period.
2. A lag period in reflectance loss was noted at temperatures below 250° F. The character of this curve is not known with certainty.
3. The data obtained are applicable to the color evaluation of sterilization processes.

The author is indebted to Mr. Paul C. Wilbur, A. E. Pech and Dr. C. R. Stumbo for their valuable suggestions and criticism.

#### REFERENCES

- (1) BELL, R. W., AND WEBB, B. H. The Relationship between High Temperature Forewarming and the Color and Heat Stability of Evaporated Milk of Different Solids Content. *J. Dairy Sci.*, 26: 579-585. 1943.
- (2) NELSON, V. The Spectrophotometric Determination of the Color of Milk. *J. Dairy Sci.*, 31: 409-414. 1948.
- (3) TARASSUK, N. P. Effect of Oxygen on Color and Flavor of Heated Milk. *Food Inds.*, 19: 781-783. June, 1947.
- (4) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of Heat Labile Sulfides of Milk. I. Method of Determination and the Influence of Temperature and Time. *J. Dairy Sci.*, 26: 689-703. 1943.
- (5) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of Heat Labile Sulfides of Milk. II. General Origin of Sulfides and Relation to Total Sulfur, Total Nitrogen and Albumin Nitrogen. *J. Dairy Sci.*, 26: 853-867. 1943.





# EFFECT OF RAW SOYBEANS AND OF SOYBEAN OIL ON PLASMA CAROTENE AND ON VITAMIN A AS MEASURED BY ACTIVATED GLYCEROL DICHLOROHYDRIN<sup>1</sup>

R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN

*Iowa State College, Ames*

Interference with vitamin A metabolism in dairy cows fed soybeans and soybean products has been reported previously. Hauge *et al.* (3, 4) were among the first to demonstrate a factor in soybeans that suppressed the transfer of the vitamin A potency of the ration to the milk fat. These workers found that the factor could be removed from soybean oil by adsorption on activated charcoal. Although their data showed no lowering of carotene, they stated that carotene as well as the vitamin A values may be lowered by additions of large amounts of either soybeans or soybean oil to the ration. Cannon *et al.* (1) observed a bleaching effect on the milk fat of cows fed raw soybeans. Their observations were based on color comparisons rather than chemical determinations of vitamin A and carotene. Shaw *et al.* (6) recently reported the occurrence of a vitamin A deficiency in dairy calves from dams fed raw soybeans.

The effects of raw soybeans and soybean oil on the blood plasma carotene and vitamin A concentrations of lactating cows fed alfalfa hay, silage, concentrates and a carotene supplement are reported herein.

## EXPERIMENTAL

*Procedure.* In this feeding trial either 9 lb. of raw soybeans or 1.7 lb. of expeller-process soybean oil, an amount calculated to be equivalent to the oil supplied by the raw beans, were incorporated into the rations of dairy cows to test their effect on the concentrations of blood plasma carotene and vitamin A. This quantity of raw soybeans, based on previous experiments (1), was selected as the probable maximum amount that could be fed daily over a prolonged experimental period.

Six Holstein cows were divided into two comparable groups. One of three experimental rations was assigned at random to each cow of Group I; these rations were duplicated for Group II. The daily feeding schedule indicating the concentrates fed is presented in table 1.

In addition to the concentrates, all cows were fed a poor quality alfalfa hay throughout the trial. Corn silage was provided for the first 6 weeks of the experimental period, at the end of which time the supply was exhausted and alfalfa hay became the only roughage. Before the trial started it was found that the cows selected had plasma carotene levels that were

Received for publication December 9, 1947.

<sup>1</sup> Journal Paper J-1506 of the Iowa Agricultural Experiment Station. Project 692.

less than 300  $\gamma$  per 100 ml. Since it was desired to conduct the studies with cows having more plasma carotene than this, so as to allow sufficient latitude for a possible depression, each cow also received daily both before and during the trial 0.5 lb. of a carotene preparation<sup>2</sup> containing 250,000 USP units of vitamin A per pound. This 0.5 lb. of carotene preparation was mixed with 2 lb. of the basal concentrate mixture and fed each cow

TABLE 1  
*Daily concentrate feeding schedule of the three cows in each of the two groups during each period*

Cow no.	Group no.	Basal concentrate mixture <sup>a</sup> (lb.)	Materials tested (lb.)
Basal period (2 weeks)			
1947	I	14.0	.....
2470	I	13.0	.....
2379	I	15.5	.....
2210	II	14.0	.....
2392	II	15.0	.....
2472	II	14.0	.....
Experimental period (9 weeks)			
1947	I	5.0	9.0 Raw soybeans
2470	I	11.3	1.7 Soybean oil
2397	I	15.5	Control
2210	II	5.0	9.0 Raw soybeans
2392	II	13.3	1.7 Soybean oil
2472	II	14.0	Control
Cross-over period (4 weeks)			
1947	I	14.0	Control
2470	I	11.3	1.7 Soybean oil
2397	I	6.5	9.0 Raw soybeans
2210	II	14.0	Control
2392	II	13.3	1.7 Soybean oil
2472	II	5.0	9.0 Raw soybeans

<sup>a</sup> The basal concentrate mixture consisted of 250 lb. of ground yellow corn, 250 lb. crushed oats, 200 lb. linseed oil meal, 100 lb. wheat bran, 9 lb. common salt, and 16 lb. bone meal.

between the morning and evening feeding periods apart from the soybean products in order to avoid possible *in vitro* destruction of the carotene (2).

The trial was initiated with a 2-week basal period during which the plasma of each cow was characterized for its carotene and vitamin A content. An experimental period of 9 weeks followed the basal period. At the end of the 9-week experimental period the rations of the cows fed the control diet and those fed the raw soybeans were switched (table 1). This cross-over period was continued for 4 weeks. All other experimental

<sup>2</sup> "Super Carex", a carrot oil preparation taken up in a dry carrier, was obtained from Nutritional Research Associates, Inc., South Whitley, Indiana.

conditions were maintained with these two groups. The cows receiving the soybean oil, however, were continued on their starting experimental ration throughout the trial.

Venous blood samples were collected weekly from each cow and were analyzed immediately for vitamin A and carotene contents. Sufficient blood was drawn to supply duplicate 9-ml. plasma samples. Kimble's (5) procedure was used for extracting the vitamin A and carotenoids from the plasma. Five-milliliter portions of these extracts were used for determining the carotenoids. The per cent transmission readings obtained with a Coleman Universal Spectrophotometer set at 440  $m\mu$  were converted into carotene values by means of a standard curve.<sup>3</sup> A new reagent, activated glycerol dichlorohydrin (G.D.H.), was used to determine the vitamin A of the blood plasmas. G.D.H. was selected in view of the potential advantages of this colorimetric reagent (7).

For the determination of vitamin A, 12-ml. portions of the plasma extracts were placed into 50-ml. centrifuge tubes. These tubes were heated in a water bath, which at no time exceeded 65° C., to evaporate the solvent. Immediately following the removal of the solvent, the tubes were cooled to room temperature and the residue in each tube dissolved in 1.5 ml. of chloroform. One milliliter of each chloroform solution was transferred to a Coleman cuvette and 4 ml. of G.D.H. added. The contents of the cuvette were mixed by inversion and the color allowed to develop for 4 minutes in the dark at room temperature, after which the readings were made with the spectrophotometer. The transmission readings were converted into vitamin A values by means of the standard curve and then corrected for carotene interference.

*Validity of the reagent used for the determination of vitamin A.* Sobel and Werbin (8) previously have shown G.D.H. to be satisfactory for the determination of the vitamin A of fish oils. Since information on the applicability of G.D.H. for the determination of vitamin A of bovine blood plasmas was unavailable, it was necessary to ascertain its validity for the type of study reported herein.

A series of recovery studies was made on pooled samples of bovine plasmas containing from 400 to 550  $\gamma$  carotene per 100 ml. Natural vitamin A ester was added at four different levels and 95.9 to 100.0 per cent recovery was obtained using G.D.H. as the colorimetric reagent. These results indicate that vitamin A could be determined satisfactorily with G.D.H.

<sup>3</sup> A Coleman Universal Spectrophotometer, Model 11, was used for all analyses. It previously was standardized at 440  $m\mu$  with crystalline B carotene for estimating the carotenoids, and at 550  $m\mu$  with a natural vitamin A ester, PC 3 capsule, obtained from Distillation Products, Inc., for estimating the vitamin A. A carotene interference curve was plotted from data obtained by the addition of G.D.H. to crystalline B carotene in chloroform.



TABLE 2  
The effect of raw soybeans and soybean oil on plasma carotene and vitamin A

Weeks	Group I						Group II					
	Control		Raw soybeans		Soybean oil		Control		Raw soybeans		Soybean oil	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
	(γ/100 ml.)						(γ/100 ml.)					
Basal period												
1	.....	.....	343	30.0	394	37.4	367	31.2	425	30.3	446	37.6
2	307	43.2	338	37.9	422	36.3	388	42.0	442	34.8	458	39.0
Experimental period												
1	365	47.3	324	45.5	422	34.9	430	45.0	420	40.9	418	47.0
2	408	45.6	317	36.8	420	53.0	437	32.3	367	39.8	449	35.1
3	550	33.4	398	43.7	518	30.0	499	37.2	386	45.8	480	30.4
4	528	31.0	346	26.0	382	30.1	422	30.5	314	34.4	473	31.0
5	504	39.2	350	40.0	396	33.2	422	33.0	259	42.6	514	32.5
6	480	40.0	420	41.6	458	41.8	420	33.7	317	34.8	593	41.0
7	662	34.6	420	24.4	509	26.5	535	30.9	341	33.6	533	29.2
8	662	47.0	425	36.0	550	45.4	535	45.3	427	45.0	480	39.3
9	571	37.0	437	40.3	566	41.2	564	45.4	403	39.5	490	40.6
Cross-over period <sup>a</sup>												
1	427	38.1	502	26.3	599	24.7	482	27.3	461	34.7	456	22.1
2	398	25.0	542	25.8	518	21.1	427	32.1	470	26.0	446	26.5
3	432	27.8	553	39.4	542	36.6	432	40.2	490	44.7	384	31.0
4	418	31.2	533	35.5	499	28.3	391	33.9	494	34.0	434	44.1

<sup>a</sup> The cows fed soybean oil were not switched but continued on the same ration in this period.

## RESULTS AND DISCUSSION

The effects of feeding raw soybeans or soybean oil were measured by the changes that occurred in the concentrations of blood plasma carotene and vitamin A found in the cows fed these products. These changes also were compared with those that occurred in the blood plasma carotene and vitamin A of the cows fed the control ration.

In table 2 are listed the amounts of carotene and vitamin A that were found in the blood plasma of each of the cows at weekly intervals. In order to get a clearer picture of the changes that occurred in the concentration of carotene in the blood plasma of the cows under the three feeding

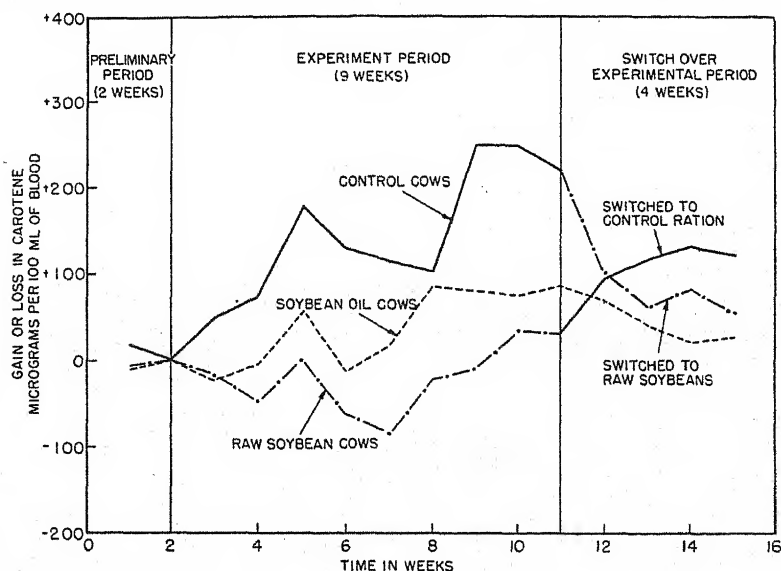


FIG. 1. Variations from the final determinations in a preliminary period, of average blood plasma carotene content for control cows, cows fed raw soybeans and cows fed soybean oil.

schedules, the data were plotted out as shown in figure 1. The zero point on these curves is the average concentration of carotene in the blood plasma at the end of the preliminary period for cows fed each feed. The changes in carotene concentration are plotted from that point.

It is apparent from the curves that the feeding of raw soybeans to cows caused their plasma carotene to decline somewhat in concentration and to remain considerably under that of cows fed the control ration. During the first 4 weeks the differences between these two lots of cows increased rapidly, then more slowly up to 7-9 weeks, when differences in concentration between the control cows and those fed raw soybeans seemed to level off at about 250  $\gamma$  carotene per 100 ml. of blood plasma.

That this difference in carotene concentration was effected by feed and not by cow differences is strongly supported by what happened when the feed of these cows was switched. Those cows formerly being fed raw soybeans and then fed the control ration showed increasing concentrations of carotene from the time the rations were changed. In opposition, those cows which were changed from the control ration to raw soybeans showed a constantly decreasing carotene concentration in their blood plasma until it was considerably under that of the other group with which its ration was switched. Although the switch-over period lasted only 4 weeks, yet in this time the differences in plasma carotene concentration reached almost one-half the magnitude that existed in a similar period before the switch-over was made.

The changes in blood plasma carotene concentration of the cows receiving soybean oil were intermediate, lying between those of the control cows and those fed raw soybeans. Apparently the oil depressed the plasma carotene concentration but not to the extent of the raw beans. The effects of feeding the oil are not as clear as with feeding raw soybeans, since no switch-over of rations was made with these cows. It is not known whether their position in relation to the control cows would have been reversed had these rations been switched. Presumably such a result would have occurred.

As was noted in outlining the feeding procedure, changes in the kind of roughages that were fed occurred during the progress of the trial. At the end of the sixth week corn silage was eliminated from the ration and alfalfa hay fed in greater amounts. Also, during the fifth week (third week of the first experimental period) all the cows were inadvertently permitted access to fresh grass for approximately 2 hours.

These changes in feed no doubt affected the carotene intake of the cows. Since all cows were fed alike and supposedly increased their carotene intakes together, these changes should have caused no serious influence on the differences in carotene concentrations between groups. Increased or decreased intakes of carotene would cause fluctuations in the plasma carotene, but each group would be affected in the same way.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and the soybean oil rations (table 2) were small and showed no particular trends. If destruction of vitamin A was being caused by either the raw soybean or soybean oil, the physiological processes of the cows quickly replenished the supply in the blood from carotene or from liver storage. Perhaps if the carotene intake of the cows were low enough, the feeding of raw soybeans and maybe soybean oil would cause a decline in the vitamin A concentration in their blood. Hauge *et al.* (3) have specified a factor that adversely affects the vitamin A concentration in milk fat. This factor might be operative on blood plasma vitamin A.



## SUMMARY

Feeding raw soybeans in the amount of 9 lb. daily to lactating cows caused marked differences in their blood plasma concentration of carotene from that of cows fed a control ration containing no soybean products. During the first 4 weeks the differences increased rapidly, but by 7-9 weeks they seemed to level off at about 250  $\gamma$  carotene per 100 ml. of blood plasma. The reversal in blood plasma concentrations of carotene that took place after a switch-over of rations was made between the cows receiving the control and the raw soybean rations indicates that the causative factor was the feed rather than the individuality of the cows.

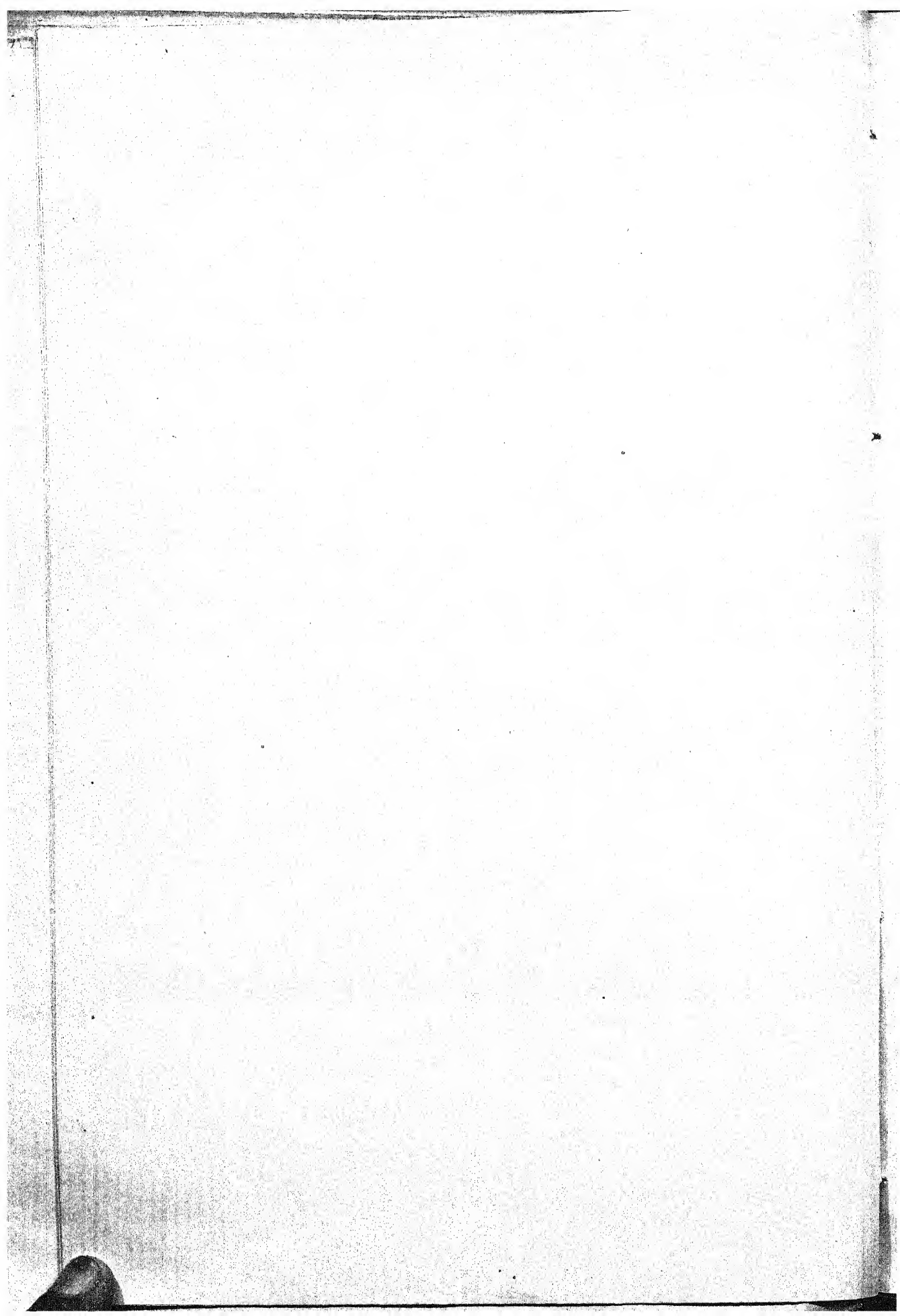
The feeding of expeller process soybean oil to lactating cows caused differences in their blood plasma concentrations that were intermediate with the concentrations found in the blood plasma of cows fed a control ration containing no soybeans or soybean products and cows fed raw soybeans. The oil apparently depressed the carotene concentrations but not to the extent of the raw soybeans.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and soybean oil rations were small and showed no particular trends.

Activated glycerol dichlorohydrin, a new reagent for the determination of vitamin A, proved to be satisfactory for this determination in bovine blood.

## REFERENCES

- (1) CANNON, C. Y., WILLIAMS, N. K., AND ESPE, D. L. Cows Are a Good Bean Market. *Farm Sci. Reporter*, 3: 14-16. 1942.
- (2) FREY, C. W., SHULTZ, A. S., AND LIGHT, R. F. The Effect of Active Soybeans on Vitamin A. *Ind. Eng. Chem.*, 28: 1254. 1936.
- (3) HAUGE, S. M., HILTON, J. H., AND WILBUR, J. W. The Absorption of the Vitamin A Suppressing Factor from Soybean Oil. *J. Dairy Sci.*, 23: 719-723. 1940.
- (4) HAUGE, S. M., WILBUR, J. W., AND HILTON, J. H. A Further Study of the Factor in Soybeans Affecting the Vitamin A Value of Butter. *J. Dairy Sci.*, 20: 87-91. 1937.
- (5) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, 24: 1055-1065. 1939.
- (6) SHAW, J. C., MOORE, L. A., AND SYKES, J. F. Vitamin A Deficiency in Dairy Cattle on Rations Containing Ground Raw Soybeans. *J. Dairy Sci.*, 30: 567. 1947.
- (7) SOBEL, A. E., AND WERBIN, H. Activated Glycerol Dichlorohydrin a New Colorimetric Reagent for Vitamin A. *Ind. Eng. Chem., Anal. Ed.*, 18: 570. 1946.
- (8) SOBEL, A. E., AND WERBIN, H. Determination of Vitamin A in Fish Liver Oils with Activated Glycerol Dichlorohydrin. *Ind. Eng. Chem., Anal. Ed.*, 19: 107. 1947.



# SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. I. GROWTH, BLOOD COMPOSITION, AND COST<sup>1</sup>

J. T. REID,<sup>2</sup> G. M. WARD, AND R. L. SALSBUURY<sup>3</sup>

*New Jersey Agricultural Experiment Station, Sussex*

In a study of the relative value of a simple and a complex concentrate mixture for young breeding bulls, the composition of the whole blood and of the plasma was investigated on the chance that the feeds might reflect different physiological effects upon these tissues. Quantitative studies of various blood constituents have proved invaluable in experimental, diagnostic and clinical work, despite the variability in "normals" observed in different individuals, sexes, species, physiological functions, regions, seasons and climates. Most studies of bovine blood composition have concerned the female rather than the male.

Table 1 summarizes the levels of several constituents of whole blood and plasma of bulls as found in previous studies.

Since the literature involving the relationships of whole blood and plasma constituents of cattle to diet, age, physiological functions and pathological conditions is too extensive to be considered here, recognition has been given only to those data which reflect the blood picture of healthy bulls and which are pertinent to the present study.

The purpose of this study was to ascertain the effects of a simple and a complex concentrate mixture upon growth as determined by wither height and heart girth measurements and upon the concentration of some of the constituents of blood. The cost of maintaining breeding bulls on these feeding regimes was examined.

## EXPERIMENTAL PROCEDURE

Sixteen Holstein bulls of similar blood lines were obtained at birth and reared under the same management and feeding regime until the commencement of the experiment. From these animals the 12 bulls used in this investigation were selected at 18 months of age on the basis of uniformity of age, size, and blood and semen pictures. The 12 bulls composed two groups of six each. Group I received a simple concentrate mixture of

Received for publication December 15, 1947.

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Industry.

<sup>2</sup> Present address, Department of Animal Husbandry, Cornell University, Ithaca, New York.

<sup>3</sup> The authors are indebted to Dr. Stacy B. Randle, state chemist, for a portion of the feed analyses and to Prof. C. E. Shuart and Messrs. M. Struble and D. Eby for the care and feeding of the animals.



TABLE 1  
Summary of previous studies on levels of several constituents of whole blood and plasma in bulls

Investigator	No. of animals	Age	Whole blood			Blood plasma			
			Hb	R.B.C.C. <sup>a</sup> (mill./mm. <sup>3</sup> )	R.B.C.V. <sup>b</sup> (%)	Ca (mg. %)	Inorg. P (mg. %)	Total proteins (%)	Ascorbic acid (mg. %)
Aberhalden (1)	1	24 mo.	10.64	.....	33.43	7.93	2.73	6.97	.....
Kusner (12)	.....	.....	11.74	8.24	.....	.....	.....	.....	.....
Knoop <i>et al.</i> (11)	6	2 days-37 wk.	10.52	9.78	.....	.....	.....	.....	.....
Anderson <i>et al.</i> (2)	5	1-5 mo.	11.81	.....	.....	.....	.....	.....	.....
.....	5	birth to 10 mo.	.....	.....	.....	12.62	3.34	.....	.....
McGey (15)	6	Mature	12.80	.....	.....	.....	.....	.....	.....
Brook and Hughes (5)	24	.....	11.92	.....	.....	.....	.....	.....	.....
Dimock and Thompson (7)	3	.....	10.66	5.81	.....	.....	.....	.....	.....
Lamarre (13)	4	.....	.....	6.50-10.90	.....	10.46	7.30	.....	.....
Payne <i>et al.</i> (18)	45	12 mo.	.....	.....	.....	13.03	4.76	.....	.....
.....	27	Over 12 mo.	.....	.....	.....	.....	.....	7.28	.....
Schwob (24)	.....	.....	.....	.....	.....	.....	.....	.....	0.19-0.39
Bortree <i>et al.</i> (3)	10	.....	.....	.....	.....	.....	.....	.....	.....
Hamersma (9)	1	26 mo.	12.79	.....	.....	11.40	7.2	.....	.....
.....	1	27 mo.	12.18	.....	.....	10.60	7.5	.....	.....
Phillips <i>et al.</i> (19)	22	.....	.....	.....	.....	.....	.....	.....	0.27

<sup>a</sup> Red blood cell count.  
<sup>b</sup> Red blood cell volume.

which corn and corn gluten meal constituted a large portion, while Group II was fed a complex concentrate mixture (table 2). Both groups received the same average grade timothy-clover hay. The average composition of the concentrate mixtures and hay used during the feeding trial is shown in table 3.

The bulls were fed 1 lb. of hay per 100 lb. body weight daily with concentrate feed in sufficient quantity to provide an average daily digestible nutrient intake of approximately 1.02 and 1.18 lb. per 100 lb. body weight before and after an average age of 760 days, respectively. Feed intake was adjusted at average intervals of 43 days following heart girth and

TABLE 2  
*Composition of concentrate mixtures*

Ingredients	Group I	Group II
	(%)	(%)
Ground yellow corn .....	54.0	10.0
Beet pulp .....	25.0	.....
Corn gluten meal .....	10.0	.....
Cane molasses .....	10.0	10.0
Linseed meal .....	.....	12.0
Soybean meal .....	.....	17.0
Crushed oats .....	.....	25.0
Wheat bran .....	.....	10.0
Dehydrated alfalfa .....	.....	10.0
Limestone .....	.....	2.0
Iodized salt .....	1.0	1.0
Bone meal .....	.....	0.7
Brewers yeast .....	.....	1.95
Mineral salt mixture <sup>a</sup> .....	.....	0.1
Fish liver oil <sup>b</sup> .....	.....	0.2
Irradiated yeast <sup>c</sup> .....	.....	0.05
	100.00	100.00

<sup>a</sup> Mineral salt mixture consisted of:  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 50%;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 44.5%;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 5.0%; and  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 0.5%.

<sup>b</sup> Fish liver oil containing 15,000 I.U. or more of vitamin A per g.

<sup>c</sup> Irradiated yeast containing 9,000 USP units of vitamin D per g.

with height measurements. The average daily digestible nutrient intake immediately following body measurement was 1.08 and 1.22 lb. per 100 lb. body weight before and after an average age of 760 days, respectively, and regressed to about 0.96 and 1.11 lb., respectively, before the next adjustment as calculated from Morrison's tables (17). Body weight was calculated from the heart girth measurement according to the equation suggested by Branton and Salisbury (4).

An attempt was made to obtain about the same amount of semen from the bulls of each group; however, more was being taken from Group II during the latter part of the experiment than from Group I. The average daily semen volume and accumulative semen volume taken from the two groups are represented by the graphs and curves, respectively, shown in

TABLE 3  
Average chemical composition of feeds (per cent of dry matter)

Group	Protein	Fat	Fiber	Ash	N.F.E.	P	Ca	Mn
Concentrate mixtures								
I	12.00	2.72	8.70	4.73	64.47	0.30	0.33	0.0044
II	21.76	4.67	10.08	9.03	49.03	0.66	0.97	0.0233
Hay								
	6.44	2.24	38.73	4.85	44.02	0.15	0.30	0.0053

figure 1, heart girth measurements also being included. The difference in the accumulative semen volume does not indicate that the bulls of Group II were capable of producing greater volumes of semen but merely that more semen was taken from these animals than from the bulls of Group I.

No data were obtained on the semen of one bull in Group II, since this animal manifested a fear which precluded obtaining semen from him in the usual manner. One bull was eliminated from Group I about mid-trial because of tuberculosis reaction. The animals were 18 months old at the beginning and 33 months old at the termination of the experiment. Twenty days was the greatest difference between the ages of any of the bulls.

The data on the phase of the study dealing with the blood constituents have been grouped into 3-month age periods for convenience of study. The

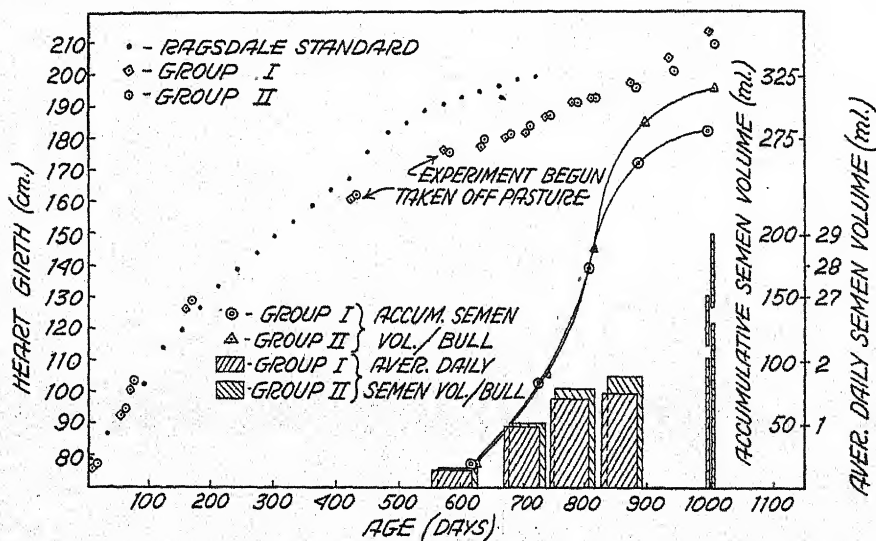


FIG. 1. Heart girth measurements of bulls, the Ragsdale standard (20) for Holstein bulls, and the average daily and accumulative volumes of semen produced per bull.



chemical methods used to determine the levels of certain blood and plasma constituents are as follows: hemoglobin, Sanford *et al.* (23); glutathione, Woodward and Fry (26); calcium, Clark and Collip (6); inorganic phosphorus, Fiske and Subbarow (8); phosphatase, method of King and Armstrong (10) as modified by Wiese *et al.* (25); plasma proteins, albumin and globulins, Looney and Walsh (14); and ascorbic acid, modification of method of Mindlin and Butler (16). The red blood cell count and volume (hematocrit) were determined on the same blood samples according to the standard procedures.

#### RESULTS

*Growth.* Good growth of bulls was effected by both feeding regimes once the retarded growth which was incurred on the late fall pasture

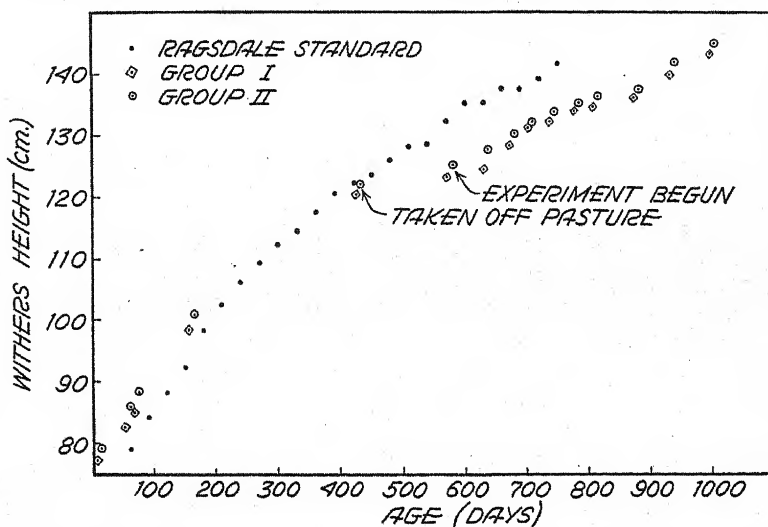


Fig. 2. Withers height measurements and the Ragsdale standard (20) for Holstein bulls.

prior to the commencement of the feeding trials was overcome (figs. 1 and 2). Semen production did not appear to affect growth (fig. 1). A study of the body measurements of the bulls of both groups would indicate a slightly more rapid growth of these animals during the actual feeding trial than is considered standard for Holstein bulls of the same age (20). It should be pointed out, however, that the standard proposed by Ragsdale (20) is based upon the measurements of only two bulls subsequent to 540 days of age. Group I animals gained body weight at an average rate of 0.18 lb. per day faster than Group II bulls. The rate of increase in height at the withers was similar for both groups (Group I, 1.57 and Group II, 1.54 mm. per day), as shown in figure 2. A similar general appearance and degree of fleshiness was observed in the animals of both groups.

TABLE 4  
Blood constituents at various ages

Constituent	Group <sup>a</sup>	Age (mo.)									
		18-21		21-24		24-27		27-30		30-33	
		No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content
Hb (gm.%)	I	12	12.07 ± 0.31	18	12.70 ± 0.29	23	13.50 ± 0.25	10	14.16 ± 0.31	15	14.49 ± 0.21
	II	12	12.32 ± 0.26	18	13.15 ± 0.16	24	13.40 ± 0.13	12	14.09 ± 0.13	18	15.16 ± 0.16
R.B.C.C. <sup>b</sup> (mill./mm. <sup>3</sup> )	I	12	9.88 ± 0.38	18	9.56 ± 0.35	23	8.88 ± 0.25	10	8.85 ± 0.47	15	8.83 ± 0.31
	II	12	9.98 ± 0.36	18	9.62 ± 0.24	24	8.62 ± 0.18	12	8.55 ± 0.25	18	9.36 ± 0.22
R.B.C.V. <sup>c</sup> (%)	I	12	35.98 ± 1.60	18	33.59 ± 0.88	23	36.67 ± 0.78	10	37.74 ± 1.03	15	40.12 ± 0.77
	II	12	35.76 ± 1.17	18	34.45 ± 0.52	24	35.58 ± 0.49	12	37.54 ± 0.86	18	40.26 ± 0.78
Mean corpuscular Hb (yy)	I	12	12.22	18	13.28	23	15.20	10	16.00	15	16.41
	II	12	12.34	18	13.67	24	15.55	12	16.48	18	16.20
Mean corpuscular volume (μ <sup>3</sup> )	I	12	36.42	18	35.14	23	41.30	10	42.64	15	45.44
	II	12	35.83	18	35.81	24	41.28	12	43.91	18	43.01
Reduced glutathione (mg.%)	I	12	28.66 ± 0.66	17	30.17 ± 1.20	23	34.79 ± 1.05	10	35.51 ± 2.12	15	41.12 ± 1.47
	II	12	27.47 ± 1.38	18	31.22 ± 1.35	24	35.06 ± 1.02	12	34.40 ± 1.45	18	41.86 ± 1.21
Oxidized glutathione (mg.%)	I	12	4.94 ± 0.53	17	8.84 ± 0.47	.....	.....	.....	.....	10	5.60 ± 0.83
	II	12	6.54 ± 0.57	18	7.99 ± 0.49	.....	.....	.....	.....	12	6.95 ± 0.43
Total glutathione (mg.%)	I	12	33.60 ± 0.71	17	39.01 ± 1.18	.....	.....	.....	.....	10	45.27 ± 2.13
	II	12	34.01 ± 1.45	18	39.21 ± 1.24	.....	.....	.....	.....	12	48.64 ± 1.64

- <sup>a</sup> Group I received simple concentrate mixture and mixed hay.  
Group II received complex concentrate mixture and mixed hay.  
<sup>b</sup> Red blood cell count.  
<sup>c</sup> Red blood cell volume.

TABLE 5  
Plasma constituents at various ages (mean and standard error)

Constituent	Group <sup>a</sup>	Age (mo.)									
		18-21		21-24		24-27		27-30		30-33	
		No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content
Ca (mg.%)	I	12	8.79 ± 0.22	18	10.49 ± 0.15	23	10.60 ± 0.10	10	11.02 ± 0.16	10	11.65 ± 0.38
	II	12	9.37 ± 0.22	18	10.09 ± 0.11	24	10.37 ± 0.09	12	10.61 ± 0.09	12	11.52 ± 0.74
Inorg. P (mg.%)	I	12	8.26 ± 0.18	18	7.76 ± 0.26	23	7.61 ± 0.21	10	7.13 ± 0.14	15	7.16 ± 0.24
	II	12	7.56 ± 0.24	18	7.78 ± 0.13	24	7.23 ± 0.26	12	6.86 ± 0.26	18	7.34 ± 0.18
Phosphatase, acid (units/100 ml.)	I	.....	.....	.....	.....	.....	.....	5	2.32 ± 0.18	10	1.43 ± 0.29
	II	.....	.....	.....	.....	.....	.....	6	1.72 ± 0.20	12	1.36 ± 0.19
Phosphatase, alkaline (units/100 ml.)	I	.....	.....	.....	.....	5	8.06 ± 0.39	10	6.09 ± 0.59	10	11.18 ± 0.56
	II	.....	.....	.....	.....	6	9.53 ± 1.81	12	7.61 ± 1.67	12	12.15 ± 0.88
Total proteins (g.%)	I	6	7.55 ± 0.06	18	6.81 ± 0.10	23	7.35 ± 0.07	10	6.80 ± 0.13	10	6.90 ± 0.10
	II	6	7.68 ± 0.17	18	6.99 ± 0.07	24	7.21 ± 0.08	12	6.90 ± 0.10	12	6.90 ± 0.07
Albumin (g.%)	I	.....	.....	.....	.....	5	2.91 ± 0.13	5	5.21 ± 0.08	9	4.05 ± 0.14
	II	.....	.....	.....	.....	6	4.14 ± 0.36	6	5.18 ± 0.13	12	4.02 ± 0.16
Globulins (g.%)	I	.....	.....	.....	.....	6	4.76 ± 0.25	5	1.82 ± 0.12	14	2.65 ± 0.14
	II	.....	.....	.....	.....	6	3.31 ± 0.35	6	1.93 ± 0.14	18	2.71 ± 0.11
Ascorbic acid (mg.%)	I	12	0.20 ± 0.01	18	0.28 ± 0.03	23	0.32 ± 0.02	10	0.37 ± 0.02	15	0.27 ± 0.02
	II	12	0.26 ± 0.02	18	0.27 ± 0.02	24	0.29 ± 0.01	12	0.34 ± 0.03	18	0.24 ± 0.01

<sup>a</sup> Group I received simple concentrate mixture and mixed hay.  
Group II received complex concentrate mixture and mixed hay.



*Blood composition.* Remarkably similar values were found for various constituents and characteristics of the blood and plasma of the animals of both groups during the same age period. These data are summarized by groups in tables 4 and 5. Since no appreciable group differences were observed, the average data from both groups would seem to be normal for bulls of similar age. Although diet did not appear to influence the composition of the blood of these animals, various trends were observed which appeared to be associated with aging.

The concentration of hemoglobin, the red blood cell volume, and the mean corpuscular hemoglobin and volume gradually increased, whereas the number of erythrocytes decreased very little as age progressed from 18 to 33 months. Other constituents tending to increase with age were plasma calcium and reduced and total glutathione. The plasma level of inorganic phosphorus tended to decrease, whereas no definite relationship between the plasma concentration of ascorbic acid and age was observed.

The variations in alkaline plasma phosphatase were attributed to the rate of semen collection as reported previously (22). Likewise, the fluctuation in the levels of albumin and globulin may have been related to the production of semen.

Data on total and oxidized glutathione are not given for the periods 24 to 27 months and 27 to 30 months because estimations of this compound could not be obtained. Inability to measure this compound was concomitant with an increased rate of semen collection and appeared to be caused by a factor(s) existing in blood plasma under these conditions which prevented the reduction of oxidized glutathione by metallic zinc (21).

*Cost.* A comparable gain in body weight cost approximately 50 per cent more in Group II than in Group I. The average cost of maintaining a bull on the Group II regime was \$52.75 more per year than that of a bull receiving the other diet.

#### DISCUSSION

No important differences were found in the growth, general health, and blood constituents of two groups of breeding bulls receiving markedly different concentrate feeds. On the basis of these criteria, it would seem that costly, complex concentrate mixtures are not necessary for animals of similar age and producing semen at similar rates. The final evaluation of complex feeds, however, necessarily lies in their effects upon the production of fertile semen. A subsequent report in this series will consider the production of semen by the bulls used in this study.

Although this investigation revealed no group differences, various trends appeared to be associated with aging. In view of the lack of dietary influence, the concentration of some blood constituents investigated in this study would appear to be standard for bulls of similar age and breed when maintained under climatic conditions similar to those of northern New Jersey (tables 4 and 5).

The results of this study would seem to indicate the importance of the rumen in the nutrition of the bull. Regardless of the supposed limitations of the simple concentrate feed received by Group I animals, these bulls were able to maintain, at levels similar to those of bulls receiving a more complex diet, not only growth but also blood constituents believed to be indicative of physical well being. It should be pointed out that these relationships may not necessarily hold for bulls of greater age or for the same bulls over a longer period of time, as these data were obtained from bulls during the interval of 18 to 33 months of age. The importance of the poor to average grade hay fed to both groups may be underestimated in these considerations. Since hay and concentrates were fed in a manner believed to be consistent with good feeding practice, and since the same hay was fed to both groups, the main considerations involved comparisons of the effects of the two concentrate mixtures. Additional data dealing with the merits of these diets are presented in the subsequent paper on semen production of young bulls.

Although the cost of maintaining a sire by the ordinary breeder is of no great significance if satisfactory performance is being obtained, large bull studs such as those used in some artificial breeding units would effect a considerable saving by using simple concentrate mixtures similar to the one employed in this study rather than complex, high protein mixtures.

#### SUMMARY

1. Comparable rates of growth and concentrations of several blood constituents were found in bulls receiving a simple and a complex concentrate mixture.
2. Since the levels of certain blood and plasma constituents were similar for the two groups, these figures are presented as standards for healthy Holstein bulls of similar age and producing semen at similar rates.
3. The hematocrit, mean corpuscular hemoglobin and volume, the level of hemoglobin, reduced and total glutathione, and plasma calcium tended to increase with aging, whereas the plasma concentration of inorganic phosphorus decreased.
4. The maintenance of bulls on the complex concentrate feed cost approximately 50 per cent more than that of bulls receiving the simple concentrate mixture.

#### REFERENCES

- (1) ABDERHALDEN, E. Zur quantitativen vergleichenden Analyse des Blutes. *Z. physiol. Chem.*, 25: 5-115. 1898.
- (2) ANDERSON, A. K., GAYLEY, H. E., AND PRATT, A. D. Studies of the Chemical Composition of Bovine Blood. *J. Dairy Sci.*, 13: 336-348. 1930.
- (3) BORTREE, A. L., HUFFMAN, C. F., AND DUNCAN, C. W. Ascorbic Acid Stimulation in the Blood Plasma of Dairy Cattle Produced by the Ingestion of Chlorobutanol. *J. Dairy Sci.*, 26: 553-562. 1943.
- (4) BRANTON, C., AND SALISBURY, G. W. The Estimation of the Weight of Bulls from Heart Girth Measurements. *J. Dairy Sci.*, 29: 141-143. 1946.

- (5) BROOK, H. J., AND HUGHES, J. S. The Hemoglobin Content of the Blood of Dairy Cattle. *J. Nutrition*, 5: 35-38. 1932.
- (6) CLARK, E. P., AND COLLIP, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification. *J. Biol. Chem.*, 63: 461-464. 1925.
- (7) DIMOCK, W. W., AND THOMPSON, M. C. Clinical Examination of the Blood of Normal Cattle. *Am. Vet. Rev.*, 30: 553-559. 1906.
- (8) FISKE, C. H., AND SUBBAROW, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, 66: 375-400. 1925.
- (9) HAMERSMA, P. J. Chemical Blood Studies. VII. Onderstepoort. *J. Vet. Sci. An. Husb.*, 8: 441-483. 1937.
- (10) KING, E. J., AND ARMSTRONG, A. R. Convenient Method for Determining Serum and Bile Phosphatase Activity. *Can. Med. Assoc. J.*, 31: 376-381. 1934.
- (11) KNOOP, C. E., KRAUSS, W. E., AND WASHBURN, R. G. The Development of Nutritional Anemia in Dairy Cattle. *J. Dairy Sci.*, 18: 337-347. 1935.
- (12) KUSNER, H. E. Sostav krovi krupnogo rogatogo skota v svjazi s ego produktivnostju. *Trud. Inst. Genet. (Mosk.)*, 13: 95-120. 1940. (Abs., *Animal Breeding Abstracts*, 10: 155-156. 1942.)
- (13) LAMARRE, L. Semeiologie du sang des bovides. *Rec. méd. vét.*, 109: 69-96. 1933. (Abs., *Biol. Abstracts*, 10: 5437. 1936.)
- (14) LOONEY, J. M., AND WALSH, A. I. The Determination of Globulin and Albumin in Blood Serum by the Photoelectric Colorimeter. *J. Biol. Chem.*, 130: 635-639. 1939.
- (15) MCCAY, C. M. The Hemoglobin and Total Phosphorus in the Blood of Cows and Bulls. *J. Dairy Sci.*, 14: 373-378. 1931.
- (16) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma; A Macromethod and Micromethod. *J. Biol. Chem.*, 122: 673-686. 1938.
- (17) MORRISON, F. B. Feeds and Feeding. 20th Ed. Morrison Publishing Company, Ithaca, New York. 1939.
- (18) PAYNE, M. G., CLARK, A. G., KINGMAN, H. E., AND STANSBURY, W. M. Blood Levels of Calcium and Inorganic Phosphorus in Hereford Cattle. *J. Agr. Research*, 72: 357-363. 1946.
- (19) PHILLIPS, P. H., LARDY, H. A., BOYER, P. D., AND WERNER, G. M. The Relationship of Ascorbic Acid to Reproduction in the Cow. *J. Dairy Sci.*, 24: 153-158. 1941.
- (20) RAGSDALE, A. C. Growth Standards for Dairy Cattle. *Mo. Agr. Expt. Sta. Bull.* 336. 1934.
- (21) REID, J. T., SALSBUURY, R. L., AND WARD, G. M. Unpublished data. 1947.
- (22) REID, J. T., WARD, G. M., AND SALSBUURY, R. L. The Relationship of Semen Production, Age, Plasma Calcium and Plasma Phosphorus to the Plasma Phosphatases of Dairy Bulls. (Abstract) *J. Dairy Sci.*, 30: 546-547. 1947.
- (23) SANFORD, A. H., SHEARD, C., AND OSTERBERG, A. E. The Photometer and Its Use in the Clinical Laboratory. *Am. J. Clin. Path.*, 3: 405-420. 1933.
- (24) SCHWOB, L. Uber den Gesamteiweissgehalt und das Albumin-Globulin-verhältnis in normalen und pathologischen Pferde, Rinder und Kalberseren. Dissertation, University of Zurich, 1932. (Abs., *Biol. Abstracts*, 9: 5111. 1935.)
- (25) WIESE, A. C., JOHNSON, B. C., ELVEHJEM, C. A., HART, E. B., AND HALPIN, J. G. A Study of Blood and Bone Phosphatase in Chick Perosis. *J. Biol. Chem.*, 127: 411-420. 1939.
- (26) WOODWARD, G. E., AND FRY, E. G. The Determination of Blood Glutathione. *J. Biol. Chem.*, 97: 465-482. 1932.



## SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. II. SEMEN PRODUCTION<sup>1</sup>

J. T. REID,<sup>2</sup> G. M. WARD, AND R. L. SALSBUURY<sup>3</sup>

*New Jersey Agricultural Experiment Station, Sussex*

The comparative merits of simple and complex concentrate mixtures for semen production by bulls have not been ascertained previously. Various investigations, however, have demonstrated the efficacy and the lack of effect of certain feeds and specific nutritional factors upon the quantity and quality of semen ejaculated by bulls. Jones *et al.* (12) showed that rations which are satisfactory for normal growth to 3 years of age are adequate for normal reproductive performance. Recent Cornell investigations (6, 26) in which total digestible nutrient levels of 100, 120, and 140 per cent of the Morrison dry cow maintenance requirements (21) were fed to breeding bulls demonstrated that neither the quantity and quality of semen produced nor the fertility of the bulls was related to the digestible nutrient intake within the limits studied. Concentrate mixtures containing 12, 16, and 20 per cent total protein did not affect significantly the fertility of bulls (6, 26). However, bulls receiving the 20 per cent protein concentrate produced significantly greater concentrations of spermatozoa and lower ejaculate volume and motility and less total spermatozoa per ejaculate than did bulls receiving the other concentrate mixtures. For bulls in active service, these workers (6, 26) suggested feeding at the rate of 1 lb. of hay and 0.4 to 0.5 lb. concentrate mixture containing 12 per cent protein per 100 lb. body weight daily.

Jones *et al.* (11) found that bulls fed alfalfa hay supplemented with 1 lb. each of skim milk powder and oats groats daily grew faster, matured earlier, were in better condition, and produced good quality semen earlier than bulls receiving a basal ration of hay supplemented with salt, phosphorus and iodine. These differences were attributed to the greater energy intake rather than to the quality or quantity of protein ingested.

Since it was not possible in this experiment to use the semen from the unregistered bulls for breeding purposes, a number of measures of semen quantity and quality were employed as criteria of the relative merits of the two concentrate mixtures fed. Numerous reports support the use of the following tests of semen quality and quantity as an evaluation of rela-

Received for publication December 15, 1947.

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Industry.

<sup>2</sup> Present address, Department of Animal Husbandry, Cornell University, Ithaca, New York.

<sup>3</sup> The authors are indebted to Prof. C. E. Shuart, Messrs. M. Struble and D. Eby for the care and feeding of the animals.

tive fertility: spermatozoa concentration (15, 16, 23, 29, 32, 34); initial motility (9, 15, 16, 20, 28, 30, 31); livability or maintenance of motility (1, 10, 17, 20, 30, 31, 34); pH (2, 3, 4, 8, 9, 10); change in pH upon incubation (1, 4, 16); number of morphologically abnormal spermatozoa (1, 10, 15, 19, 32, 36); semen and spermatozoa volume (1, 3, 7, 16, 32); semen level of ascorbic acid (8, 13, 14, 22); and reducing capacity (5, 27, 28, 33).

It was the purpose of this study to evaluate the relative merits of a simple and a complex concentrate mixture for the production of semen by young bulls as determined by various tests for semen quantity and quality.

#### EXPERIMENTAL PROCEDURE

A study of the semen obtained from the two groups of bulls (one receiving a simple concentrate mixture and the other a complex mixture) was made simultaneously with the investigation of the blood composition, growth, and cost of maintenance of these animals reported in the first paper of this series (25). For details on the composition of feeds, rate of feeding, age and growth of the bulls, and the cost of the concentrate feeds, the reader is referred to the previous paper (p. 429). Of the 12 animals composing the two groups (reported in the previous paper), five in each group yielded semen during the entire experiment.

Semen quantity and quality were studied during four periods ranging from 56 to 65 days in length, separated by rest periods of 14 to 51 days, as shown in table 1. These collection periods loosely represent the four seasons.

The quantity of semen produced was expressed as the number of ejaculates obtained, the average ejaculate volume, and the average daily semen volume per bull. The quantity of spermatozoa was determined by direct counts using a cytometer and by centrifugation of semen in hematocrit tubes. These data were expressed as the concentration of spermatozoa, the proportion of the whole semen volume consisting of spermatozoa, and the average size of a spermatozoan in terms of volume (cubic micra).

Since a constant temperature stage incubator was found to be necessary but was not obtainable during the first period, no attempt was made to procure data on motility and allied characteristics for this period. During subsequent periods, the initial motility, the motility at intervals following the initial estimation, and the livability of spermatozoa were determined by means of a constant temperature stage incubator adjusted to a temperature of 100° F. The data on motility are expressed in terms of arbitrary units derived from separate estimations of the number of living spermatozoa and the spermatozoa engaged in progressive motility. The motility units used here would be approximately equivalent to motility data expressed in terms of per cent  $\div$  5. Livability of spermatozoa was calculated as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

TABLE 1  
*Semen characteristics and constituents*

Period no.		I	II	III	IV	Summary <sup>b</sup>
Period date		3/5-5/9/46	6/29-8/28/46	9/12-11/14/46	12/5/46-1/30/47	3/5/46-5/22/47
Av. beginning and terminal ages of bulls	I <sup>a</sup> II <sup>a</sup>	556-621 564-629	672-732 680-740	747-810 755-818	831-887 839-895	556-998 564-1006
Volume	Av. ejaculate volume (ml.)	I 2.95(40) <sup>c</sup> II 3.18(33)	3.52(110) 3.79(91)	3.70(144) 4.15(120)	4.13(101) 4.44(111)	3.82(423) 4.19(383)
	Av. daily semen volume/bull (ml.)	I 0.30 II 0.32	1.07 1.15	1.41 1.58	1.49 1.76	..... .....
	Av. spermatozoa conc. (mill./mm. <sup>3</sup> )	I 0.735(25) II 0.775(23)	0.891(41) 0.962(34)	1.026(52) 1.020(45)	0.823(47) 1.087(47)	0.869(193) 0.950(177)
	Av. % spermatozoa <sup>d</sup>	I ..... II ..... ..... .....	6.62(31) 6.54(24)	7.44(51) 7.48(45)	9.24(42) 9.56(42)	7.84(124) 8.06(111)
Spermatozoa	Av. spermatozoan volume (μ <sup>3</sup> )	I ..... II ..... ..... .....	84.03(30) 72.86(24)	78.22(51) 77.63(45)	105.26(42) 90.63(42)	88.87(123) 81.52(111)
	Initial motility <sup>e</sup>	I ..... II ..... ..... .....	13.31(36) 12.76(29)	13.17(52) 13.29(45)	11.05(47) 12.72(47)	11.98(163) 12.61(149)
	Motility at 100 hours <sup>f</sup>	I ..... II ..... ..... .....	2.55(34) 2.49(27)	5.65(52) 4.51(45)	7.54(47) 7.77(46)	5.53(133) 5.32(118)
	Livability at 100 hours <sup>g</sup>	I ..... II ..... ..... .....	17.23(34) 19.68(27)	35.46(52) 31.82(45)	58.49(47) 52.93(46)	38.94(133) 37.27(118)
Reducing substances	Total reducing substances (mg.%)	I 30.32(19) II 33.55(17)	37.30(41) 37.94(34)	36.48(47) 41.15(41)	26.47(21) 34.79(25)	34.18(128) 37.75(117)
	Reducing substances in oxidized state (mg.%)	I 15.89(13) II 18.40(10)	14.07(41) 13.55(33)	13.55(38) 15.13(33)	5.84(21) 6.83(23)	12.58(113) 13.01(99)
	Potential reducing capacity (mg.%)	I 43.21(15) II 53.59(12)	51.37(41) 50.73(33)	50.04(38) 56.05(33)	32.32(21) 39.26(23)	46.39(115) 50.19(101)
	Ascorbic acid (mg.%)	I 7.01(23) II 8.59(22)	8.22(41) 7.96(34)	8.28(47) 9.32(40)	5.17(19) 7.08(23)	7.58(130) 8.36(119)
	Initial pH	I 6.86(16) II 6.77(14)	6.55(34) 6.57(28)	6.68(50) 6.59(45)	6.70(25) 6.61(25)	6.67(138) 6.62(123)
	Post incubation pH	I 6.56(16) II 6.56(14)	6.13(34) 6.19(28)	6.28(50) 6.33(45)	6.45(25) 6.41(25)	6.32(138) 6.35(123)

<sup>a</sup> Roman numerals represent group number.

<sup>b</sup> Summary includes data obtained during entire feeding experiment. (Therefore, data on semen obtained in a special study made of semen phosphatases subsequent to Period IV are included.)

<sup>c</sup> Figures in parentheses indicate number of samples studied.

<sup>d</sup> Represents proportion of total semen volume consisting of spermatozoa.

<sup>e</sup> Estimated at 100° F. Motility value × 5 is approximately equivalent to per cent motility.

<sup>f</sup> Motility rating at 100 hours subsequent to ejaculation (rating × 5 is approximately equivalent to the motility expressed as per cent).

<sup>g</sup> Livability is expressed as the per cent of original motility persisting at 100 hours subsequent to ejaculation.



The reducing substances in semen were measured by a procedure similar to that outlined by Woodward and Fry (37) for the estimation of glutathione in whole blood (24).

Ascorbic acid was determined according to the method outlined by Mindlin and Butler (18).

The pH of semen was measured immediately after ejaculation and after incubation at 37° C. for 1 hour, using a Beckman pH meter equipped with a glass electrode. The decrease in pH effected by incubation was calculated from these estimations.

TABLE 2  
Percentages of various types of morphologically abnormal spermatozoa

Abnormality	Group	Period				Av. over 426 days
		I	II	III	IV	
		(%)	(%)	(%)	(%)	(%)
<i>Head</i> Pyriform	I	5.92	4.70	4.16	4.99	4.76
	II	4.49	2.31	2.05	1.94	2.48
Tapering	I	2.60	1.04	1.04	0.99	1.28
	II	2.51	0.94	0.75	0.58	1.04
Others	I	0.52	0.79	0.84	1.16	0.84
	II	0.34	0.45	0.76	0.63	0.59
<i>Midpiece</i> Filiform	I	0.31	0.46	0.44	0.22	0.37
	II	0.49	0.48	0.32	0.23	0.36
Beaded	I	0.54	1.15	1.59	1.46	1.28
	II	1.93	1.15	1.82	1.25	1.55
Others	I	1.17	2.07	5.03	8.41	4.44
	II	0.57	1.60	4.25	9.64	4.34
<i>Tail</i> Coiled	I	0.96	0.46	0.83	2.86	1.21
	II	1.26	0.94	0.63	1.60	1.03
Beaded	I	0.32	0.12	0.35	0.34	0.29
	II	0.45	0.12	0.47	0.44	0.38
Others	I	0.33	0.18	0.59	0.70	0.47
	II	0.56	0.09	0.72	0.62	0.53
Total abnormalities	I	12.67(22) <sup>a</sup>	10.97(33)	14.87(52)	21.13(30)	14.94(137)
	II	12.60(20)	8.08(27)	11.77(46)	16.93(29)	12.30(122)

<sup>a</sup> Figures in parentheses indicate number of samples studied.

Semen smears were prepared for the estimation of morphologically abnormal spermatozoa. Priority was given to the abnormalities in the order listed in table 2 (*i.e.*, a spermatozoan showing both head and tail abnormalities was registered as possessing an abnormal head) in order that the influence of an abnormal spermatozoan would be reflected but once.

#### RESULTS

The average data for several characteristics and constituents of the semen produced by both groups of bulls at intervals during the 442-day experiment are presented in tables 1 and 2.

The Group II bulls produced slightly larger ejaculates containing a greater total number of spermatozoa than did Group I bulls. No appreciable differences were found in the concentration of spermatozoa or in the proportion of semen constituted by spermatozoa ejaculated by the two groups. The difference in the average spermatozoan volume is largely the reflection of one animal in Group I.

No appreciable differences were found in the initial motility, motility at 100 hours after ejaculation, and livability of spermatozoa of the groups. The improved livability observed in both groups during the colder months may have been a seasonal effect upon this characteristic.

Similar levels of reducing substances, reducing substances in oxidized form, and ascorbic acid were found in the semen obtained from both groups. A marked decrease was observed in the level of reducing substances in oxidized state (which was reflected in the potential reducing capacity) during Period IV.

The initial pH of semen was similar for both groups; however, semen from Group I underwent a greater decrease in pH during incubation than did that of Group II.

The data in table 2 summarize the proportions of the various types of abnormal spermatozoa found in the semen from each group of bulls. Generally, the abnormalities occurred at about the same rate in both groups, with Group I spermatozoa manifesting a greater proportion of the heads of the pyriform type. It will be noted that a greater quantity of morphologically abnormal spermatozoa appeared during Periods III and IV than previously. This may have been effected by the increased rate of semen ejaculation. The differences found between the groups in total abnormalities were attributed largely to one bull in Group I, whose semen contained a characteristically high number of abnormal spermatozoa.

#### DISCUSSION

In the evaluation of the comparative merits of simple and complex concentrate feeds for breeding bulls, the final conclusion must be based upon the over-all effects of these mixtures upon the character and/or the fertility of the semen produced. Although it has been recognized that no single test presently exists which allows an adequate prediction of the relative fertility of a semen specimen, a combination of tests involving various semen properties and characteristics is believed to contribute valuable information relative to forecasting the impregnating capacity.

In general, the quality of semen produced by the two groups of bulls receiving markedly different concentrate feeds was essentially the same, as determined by various tests. The concentration of spermatozoa in the semen was similar for both groups; however, bulls receiving the complex concentrate mixture yielded ejaculates of larger volume and greater num-

bers of spermatozoa than those ejaculated by bulls consuming the simple mixture. These differences were not regarded as of great importance, since neither group of animals produced semen which was subnormal in these respects. The spermatozoa produced by bulls receiving the two diets possessed similar average degrees of motility and livability, with a slightly higher degree of livability in the semen of bulls receiving the simple mixture during Periods III and IV and in that of bulls receiving the complex feed during Period II.

Various investigators (1, 4, 16) have pointed out the usefulness of the measure of pH change during incubation as an index of semen quality, since this test affords a gross picture of the metabolic activity of spermatozoa, probably involving the effects of spermatozoa numbers, activity and chemical changes. Other studies (1, 10, 17, 20, 30, 31, 35) have demonstrated conclusively the reliability of livability or longevity estimates as forecasters of relative fertility of semen. Because of the strong evidence offered in their support, these two measures were accorded higher recognition as criteria of semen quality than the others used in this study. From this standpoint semen of similar character was produced by the bulls on both feeding programs.

The reducing properties of semen were examined previously and found to be related to the general metabolism of spermatozoa (27, 28, 33) and to states of fertility (5, 27, 28). In view of the results of these investigations, a method was devised for the analysis of semen in which reducing materials probably not measured in previous studies could be accounted for and measured as absolute quantities. No great differences were observed in the quantities of total reducing substances, reducing substances in oxidized form, potential reducing capacity, and ascorbic acid content of the semen of the two groups of bulls.

The small differences observed between the groups relative to the percentage of abnormal spermatozoa were not regarded as important, since both groups appeared to be within a safe range as determined in a very critical examination, and since these differences are explicable on the basis of the consistently high percentage of abnormal spermatozoa shown by one bull in Group I.

Apparently when sufficient energy is provided for growing, breeding bulls, a simple mixture of concentrate ingredients is equivalent to a high protein, complex mixture from the standpoint of the quality of semen produced. Satisfactory growth was found to accompany the production of good semen when the daily digestible nutrient intake was approximately 1.18 lb. per 100 lb. body weight. It is not known whether or not the same results would be found in older bulls or in the same bulls over an extended period of time.



## SUMMARY

A study was made of the relative merits of a simple and a complex concentrate mixture when fed with a poor to average grade mixed hay for the production of semen by young bulls during a 442-day experimental period.

Various analyses of 423 ejaculates yielded by the bulls receiving the simple concentrate feed and of 383 ejaculates produced by the bulls consuming the complex concentrate mixture would indicate that good quality semen of similar character resulted from the ingestion of both diets when provided at an average rate of 1.18 lb. digestible nutrients per 100 lb. body weight daily.

Although bulls consuming the complex mixture yielded slightly larger ejaculates containing more spermatozoa per ejaculate and fewer abnormal spermatozoa than those of bulls fed the simple mixture, the decrease in pH upon incubation of semen ejaculated by the latter group was greater than that of the semen produced by the bulls fed the complex concentrate feed.

Regardless of the diet fed, the concentration of spermatozoa in semen, the initial motility, the degree of livability, the size of spermatozoa, the quantity of total reducing substances, reducing substances in oxidized state, potential reducing capacity, ascorbic acid, and the initial pH of semen were similar.

## REFERENCES

- (1) ANDERSON, J. Further Investigations on the Semen of the Bull. *Vet. Record*, 53: 197-205. 1941.
- (2) ANDERSON, J. The Hydrogen-ion Concentration of the Semen of the Bull. *J. Agr. Sci.*, 32: 298-307. 1942.
- (3) ANDERSON, J. The Clinical Significance of the Hydrogen-ion Concentration of the Semen of the Bull. *Vet. Record*, 54: 317-318. 1942.
- (4) ANDERSON, J. The Change in the pH of the Semen of the Bull after Incubation. *J. Agr. Sci.*, 34: 69-72. 1944.
- (5) BECK, G. H., AND SALISBURY, G. W. Rapid Methods for Estimating the Quality of Bull Semen. *J. Dairy Sci.*, 26: 483-494. 1943.
- (6) BRANTON, C., BRATTON, R. W., AND SALISBURY, G. W. Total Digestible Nutrients and Protein Levels for Dairy Bulls Used in Artificial Breeding. *J. Dairy Sci.*, 30: 1003-1013. 1947.
- (7) BRATANOFF, K. Determinazione del Rapporto tra il Peso ed il Volume degli Elementi Morfologici (Sedimento) e la Porzione Liquida dello Sperma di Toro e di Stallone; Possibilit  da Calcolare il Numero dei Nemaspermi in Base alle Costanti Trovate. *Fecond. Artif. (Milano)*, 4: 117-126. 1946. (Abs., *Animal Breeding Abstracts*, 15: 29-30. 1947.)
- (8) DOUGHERTY, R. W., AND EWALT, H. P. Semen Studies in the Bull. *Am. J. Vet. Research*, 2: 419-426. 1941.
- (9) ELLENBERGER, H. B., AND LOHMANN, A. H. Artificial Insemination of Dairy Cattle. *Vt. Agr. Expt. Sta. Bull.* 533. 1946.

- (10) HERMAN, H. A., AND SWANSON, E. W. Variations in Dairy Bull Semen with Respect to Its Use in Artificial Insemination. Mo. Agr. Expt. Sta. Bull. 326. 1941.
- (11) JONES, I. R., DOUGHERTY, R. W., AND HAAG, J. R. Relation of Nutrition to Growth and Breeding Performance in Dairy Bulls. I. Alfalfa Hay Rations. J. Dairy Sci., 28: 311-320. 1945.
- (12) JONES, I. R., HAAG, J. R., AND DOUGHERTY, R. W. The Relation of Nutrition to Breeding Performance in Dairy Bulls. (Abstract) J. Dairy Sci., 25: 689-690. 1942.
- (13) JÜRGENS, F. Ascorbinsäuregehalt mit Sperma fruchtbarer und unfruchtbarer Bullen und Ziegenböcke. Dissertation Chem. Inst., Hannover. Abstr. in Deut. tierärztl. Wochschr., 50: 393. 1942. (Abs., Animal Breeding Abstracts, 11: 8. 1943).
- (14) KUMARAN, J. D. S. Vitamin C, Chemical Composition and the Stain Technique of Semen. Indian Vet. J., 20: 1-8. 1943.
- (15) LAGERLÖF, N. Morphologische Untersuchungen über Veränderungen im Sperma bild und den Hoden bei Bullen mit verminderter oder aufgehobener Fertilität. Acta. Path. Microbiol. Scand., Suppl., 19: 1-254. 1934. (Abs., Biol. Abstracts, 9: 7137. 1935.)
- (16) LAING, J. A. Observations on the Characteristics of the Semen in Relation to Fertility in the Bull. J. Agr. Sci., 35: 1-24. 1945.
- (17) MARGOLIN, S., BARTLETT, J. W., AND LEPARD, O. L. The Relation of Longevity to Fertility of Bull Semen. J. Dairy Sci., 26: 983-985. 1943.
- (18) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma; A Macromethod and Micromethod. J. Biol. Chem., 122: 673-686. 1938.
- (19) MOENCH, G. L., AND HOLT, H. Sperm Morphology in Relation to Fertility. Am. J. Obstet. Gynecol., 22: 199-210. 1931.
- (20) MOORE, B. H., MAYER, D. T., AND MCKENZIE, F. F. Factors Influencing Motility and Metabolism in Ram Semen. Proc. Am. Soc. Animal Production, pp. 210-215. 1940.
- (21) MORRISON, F. B. Feeds and Feeding. 20th Ed. Morrison Publishing Company, Ithaca, New York. 1939.
- (22) PHILLIPS, P. H., LARDY, H. A., HEIZER, E. E., AND RUPEL, I. W. Sperm Stimulation in the Bull through the Subcutaneous Administration of Ascorbic Acid. J. Dairy Sci., 23: 873-878. 1940.
- (23) PINCUS, G. The Eggs of Mammals. Macmillan Company, New York, N. Y. 1936.
- (24) REID, J. T., WARD, G. M., AND SALSBUURY, R. L. Unpublished data. 1947.
- (25) REID, J. T., WARD, G. M., AND SALSBUURY, R. L. Simple versus Complex Concentrate Mixtures for Young Breeding Bulls. I. Growth, Blood Composition, and Cost. J. Dairy Sci., 31: 429-438. 1948.
- (26) SALSBUURY, G. W. The Nutrition of the Dairy Herd Sire. Abstract of Cornell Nutr. Conf., pp. 18-20. 1946.
- (27) SORENSSEN, E. Ueber Verfahren zur Untersuchung von Sperma und von Flüssigkeiten für die Verdünnung des Samens. Maandschr. Dyrlaeg., 53: 84-96. 1941. (Abs., Animal Breeding Abstracts, 11: 9. 1943.)
- (28) SORENSSEN, E. The Dehydrogenating Power of Sperm Cells as a Measure for the Fertility of Sperm. (translated title) Skand. Vet. Tid., 32: 358-373. 1942. (Abs., Animal Breeding Abstracts, 12: 198-199. 1944.)
- (29) STEWART, D. L. The Examination of the Stud Bull for Soundness. Vet. Record, 57: 459-464. 1945.

- (30) SWANSON, E. W., AND HERMAN, H. A. Variations in Bull Semen and Their Relation to Fertility. *J. Dairy Sci.*, 24: 321-332. 1941.
- (31) SWANSON, E. W., AND HERMAN, H. A. The Correlation between Some Characteristics of Dairy Bull Semen and Conception Rate. *J. Dairy Sci.*, 27: 297-301. 1944.
- (32) TEUNISSEN, G. H. B. Steriliteit bij stieren. *Tijdschr. Diergeneesk.*, 71: 595-608. 1946. (Abs., *Animal Breeding Abstracts*, 15: 27. 1947.)
- (33) VAN DEMARK, N. L., MERCIER, E., AND SALISBURY, G. W. The Methylene-blue Reduction Test and Its Relation to Other Measures of Quality in Bull Semen. *J. Dairy Sci.*, 28: 121-128. 1945.
- (34) WEBSTER, W. M. Bovine Sterility in New Zealand. *Australian Vet. J.*, 8: 199-222. 1932.
- (35) WILLIAMS, W. L. The Pathology of Reproduction in Domestic Animals. The Role of the Male in the Pathology of Reproduction. *Vet. Med.*, 27: 288-302. 1932.
- (36) WILLIAMS, W. W., AND SAVAGE, A. Methods of Determining the Reproductive Health and Fertility of Bulls: A Review with Additional Notes. *Cornell Vet.*, 17: 374-385. 1927.
- (37) WOODWARD, G. E., AND FREY, E. G. The Determination of Blood Glutathione. *J. Biol. Chem.*, 97: 465-482. 1932.





# A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDRO-GUAIARETIC ACID IN DAIRY PRODUCTS. I. ITS ANTIOXYGENIC PROPERTIES IN MILK

J. W. STULL, E. O. HERREID, AND P. H. TRACY

*Illinois Agricultural Experiment Station, University of Illinois, Urbana*

The oxidized flavor is one of the most prevalent off-flavors which develop in market milk. This off-flavor may appear even though the raw milk is of the highest quality and the processing methods are carefully supervised in approved equipment.

## REVIEW OF LITERATURE

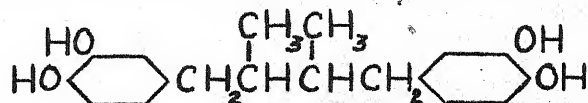
The oxidized flavor in dairy products has been studied extensively, as indicated by the voluminous amount of literature reviewed by Brown and Thurston (4), who cited 412 references.

The compounds or substances proposed as antioxidants for fats are numerous. Matill (9) studied a large number of compounds and found that the active groups of the phenolic compounds were two hydroxyl groups in either the ortho or para configuration. When these groups were in the meta position, the compound did not possess antioxidant properties.

Nordihydroguaiaretic acid (NDGA), one of the compounds which has been used as an antioxidant, was first synthesized in 1918 from hydroguaiaretic acid (12). During cooperative investigations by the United States Department of Agriculture and the University of Minnesota, NDGA was found to occur in a common desert plant, the creosote bush (*Larrea divaricata*), which grows in the southwestern United States (20). Pure NDGA is prepared by crystallization from a crude extract of the plant material.

White, crystalline NDGA is practically odorless but has a slight astringent flavor. It is only slightly soluble in water but is 50 per cent soluble in ethyl alcohol, 20 per cent soluble in propylene glycol, about 15 per cent soluble in glycerol and from 0.3 to 3 per cent soluble in fats and oils (17).

The following chemical formula has been assigned to NDGA (20):



On the basis of Matill's study (9), this phenolic compound would be expected to have antioxygenic properties since the hydroxyl groups are in the ortho position.

Extensive toxicity experiments (5) conducted for over two years indicate that NDGA is entirely harmless (1, 2) in amounts far in excess of

Received for publication January 16, 1948.

that required to prevent the oxidation of fat over extended periods of storage.

NDGA has been used successfully in retarding the development of rancidity<sup>1</sup> in lard (6, 7, 8), in bacon (16), and in salt-cured fish (15); in retarding the oxidation of esters of fatty acids (18); in stabilizing carotene in vegetable oil solutions (3, 11); and in retarding oxidative changes in vegetable oils (10) and frozen cream (19).

#### EXPERIMENTAL METHODS

The milk used in this study was produced by the University dairy herd. In cases where it was important to have milk with little or no metal contamination, the milk was taken directly from the stainless steel milking machines. In other cases, the milk was taken from aluminum milk cans after it arrived at the University Creamery.

Milk samples were scored or criticized for flavor by three or more judges and the consensus taken as the score or criticism. The judges were not aware of the history or treatment of the samples.

Vitamin C determinations were made using the rapid method of Sharp *et al.* (13).

#### RESULTS

*The concentration of NDGA needed for antioxidant protection—the effect of method of adding.* Because concentrations of 0.005 per cent were being used successfully in the treatment of fats and oils (1, 6, 7, 8, 10, 11, 15, 16, 18, 19), concentrations of 0.0075 per cent or less, expressed on the basis of the fat content of the milk, were used in this study. The NDGA was added to 4 per cent milk before it was pasteurized at 143° F. for 30 minutes. The development of the oxidized flavor was induced by adding 0.3 p.p.m. copper. The results of a representative trial are found in table 1. The trials were conducted during the period of April through August. A concentration as low as 0.00125 per cent NDGA added either in glycerol solution or in water suspension inhibited the development of the oxidized flavor during 5 days of storage at 40° F. in milk containing 0.3 p.p.m. added copper.

*The effect of NDGA on the disappearance of vitamin C in pasteurized milk.* The disappearance of vitamin C is reported by Sharp *et al.* (14) to be related to the development of the oxidized flavor in milk. For this reason a series of experiments was conducted to determine whether or not NDGA would retard the loss of vitamin C in milk under normal conditions of storage. Milk was taken directly from the milking machine and samples were prepared containing 0.00125 per cent and 0.0075 per cent NDGA

<sup>1</sup> In other branches of the food industry, the term rancidity usually is used synonymously with the term oxidation. In the dairy industry, oxidation is used to denote oxidative changes in fat, whereas rancidity characterizes hydrolytic changes.



added both in glycerine solution and in water suspension. The milk was pasteurized and cooled. Vitamin C determinations and flavor scores were made every 24 hours. The results of a representative trial are presented in table 2. The trials were conducted during the period of April through August.

The data show that NDGA retarded the destruction of Vitamin C in pasteurized milk stored at 40° F. without added copper. At the end of 96 hours of storage, all of the vitamin C had disappeared in the control samples of the milk which did not contain added copper. At the end of

TABLE 1

*The concentration of NDGA needed for antioxidant protection (storage at 40° F.)*

Sample no.	Treatment	Flavor comments				
		24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	Control + 0.3 p.p.m. cu.	Sl. cooked Sl. feed	Oxidized 1 <sup>a</sup>	Oxidized 2	Oxidized 3	Oxidized 3
3	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
6	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed

<sup>a</sup> The intensity of the oxidized flavor was given values from 1 to 5, as the level of the defect increased.

the same storage period, the loss of vitamin C in the samples which contained NDGA ranged from 54.9 per cent at the lower concentration (0.00125 per cent) to 41.9 per cent at the higher concentration (0.0075 per cent).

Even though the vitamin C disappeared in 24 to 48 hours in the milk which contained 0.3 p.p.m. added copper, the oxidized flavor did not develop during 5 days of storage in the samples which contained NDGA.

The antioxidant retarded the destruction of vitamin C during pasteurization. In the milk which contained no added copper, 18.7 per cent of the vitamin C was destroyed in the control samples during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 6.2 per cent at the higher concentration (0.0075 per cent) to 12.5 per cent at the lower concentration (0.00125 per cent).

TABLE 2  
The effect of NDGA on the disappearance of vitamin C in pasteurized milk stored at 40° F.

Sample no.	Treatment	Per cent loss of vitamin C						Flavor criticisms					
		0 hr. <sup>a</sup>	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	18.7 <sup>b</sup>	21.9	25.0	66.8	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	0.00125% NDGA	12.5	12.5	15.6	51.6	52.4	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
3	0.00125% NDGA in glycerine	12.5	12.5	12.5	45.6	54.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	0.0075% NDGA in water	6.2	6.2	15.6	47.8	41.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	0.0075% NDGA in glycerine	9.4	15.6	15.6	40.7	41.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
0.3 p.p.m. copper added													
6	Control	56.3	84.4	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Oxi- dized 1 <sup>c</sup> Sl. feed	Oxi- dized 2 Sl. feed	Oxi- dized 2 Sl. feed	Oxi- dized 4 Sl. feed
7	0.00125% NDGA in water	56.3	87.5	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
8	0.00125% NDGA in glycerine	53.2	87.5	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
9	0.0075% NDGA in water	49.9	81.2	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
10	0.0075% NDGA in glycerine	41.8	75.1	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed

<sup>a</sup> Immediately after pasteurization and cooling to 40° F.

<sup>b</sup> Vitamin C content of raw milk, 14.8 mg./l.

<sup>c</sup> The numbers 1 to 5 indicate increasing levels of oxidized flavor defect.

In the milk which contained 0.3 p.p.m. added copper, 56.3 per cent of the vitamin C was destroyed in the control sample during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 41.8 per cent at the higher concentration (0.0075 per cent) to 53.2 per cent at the lower concentration (0.00125 per cent).

There was no significant difference between the protective effect exerted by the antioxidant which was added in solution and that which was added in water suspension.

#### CONCLUSIONS

1. Concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will prevent the development of the oxidized flavor during 5 days of storage at 40° F. in whole milk containing 0.3 p.p.m. added copper.
2. In the absence of added copper, the addition of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C in whole milk stored at 40° F.
3. In the absence of added copper, concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C during pasteurization at 143° F. for 30 minutes.

#### REFERENCES

- (1) BELL, E. T. Univ. of Minn. Medical School. Personal Communication. December 6, 1946.
- (2) BELL, E. T. Univ. of Minn. Medical School. Personal Communication. July 25, 1947.
- (3) BICKOFF, E., AND WILLIAMS, K. T. Stability of Carotene at Elevated and Room Temperatures. *Oil & Soap*, 23: 65-69. 1946.
- (4) BROWN, W. C., AND THURSTON, L. M. A Review of Oxidation in Milk and Milk Products as Related to Flavor. *J. Dairy Sci.*, 23: 629-685. 1930.
- (5) CRANSTON, E. M., JENSEN, M. J., MOREN, A., BREY, T., BELL, E. T., AND BIETER, R. N. The Acute and Chronic Toxicity of Nordihydroguaiaretic Acid. *Federation Proc.*, 6: 318. 1947.
- (6) HIGGINS, J. W., AND BLACK, H. C. A Preliminary Comparison of the Stabilizing Effect of Several Recently Proposed Antioxidants for Edible Fats and Oils. *Oil & Soap*, 21: 277-279. 1944.
- (7) LUNDBERG, W. O., AND HALVORSON, H. O. Observations on Solubilities and Other Properties of Several Antioxidants in Fats. *Proc. Inst. Food Technol.*, 1945. Pp. 115-127.
- (8) LUNDBERG, W. O., HALVORSON, H. O., AND BURR, G. O. The Antioxidant Properties of Nordihydroguaiaretic Acid. *Oil & Soap*, 21: 33-36. 1944.
- (9) MATILL, H. A. Antioxidants and the Autoxidation of Fats. *J. Biol. Chem.*, 90: 141-152. 1931.
- (10) MATILL, K. F., FILER, L. J., AND LONGNECKER, H. E. A Study of the Antioxidant Effectiveness of Several Compounds on Vegetable Fats and Oils. *Oil & Soap*, 21: 160-161. 1944.
- (11) RICHARDSON, G. A., AND LONG, LOUISA M. The Use of Carotene for Coloring Butter. *J. Dairy Sci.*, 30: 533-534. 1947.



- (12) SCHROETER, G., LICHTENSTADT, L., AND IRINEU, D. Über die Konstitution der Guajacharz-Substanzen. Ber., 51: 1587-1613. 1918.
- (13) SHARP, P. F., HAND, D. B. AND GUTHRIE, E. S. Quantitative Determination of Dissolved Oxygen, Ascorbic Acid Oxidase Method. Ind. Eng. Chem., Anal. Ed., 13: 543-597. 1941.
- (14) SHARP, P. F., TROUT, G. M., AND GUTHRIE, E. S. Vitamin C, Copper and the Oxidized Flavor in Milk. Ann. Rept., N. Y. State Assoc. Dairy and Milk Inspectors, 10: 153-164. 1936.
- (15) SILVER, R. E. Retarding Rancidity in Salt Cured Fish. Food Inds., 17: 1454-1457. 1945.
- (16) SMITH, F. H., BRADY, D. E., AND COMSTOCK, R. F. Rancidity of Bacon, Effect of Antioxidants. Ind. Eng. Chem., 37: 1206-1209. 1945.
- (17) STANGE, W. J., Co., Chicago, Illinois. Unpublished Data. 1945.
- (18) STIRTON, A. J., TURER, J., AND RIEMENSCHNEIDER, R. W. Oxygen Absorption of Methyl Esters of Fat Acids and the Effect of Antioxidants. Oil & Soap, 22: 81-83. 1945.
- (19) STULL, J. W., HERREID, E. O., AND TRACY, P. H. A Study of the Use of Nordihydroguaiaretic Acid in the Storage of Frozen Sweet Cream. J. Dairy Sci., 30: 541-542. 1947.
- (20) WALLER, C. W. A Phytochemical Study of *Larrea divaricata*. Ph.D. Thesis, Univ. of Minn. 1942.

## VITAMIN D CONTENT OF ROUGHAGES<sup>1</sup>

J. A. NEWLANDER

*Vermont Agricultural Experiment Station, Burlington*

Very little vitamin D generally is considered to exist in the green growing plant. Ergosterol or some other provitamin is present and may be changed over to vitamin D when the plant is cut and exposed to the radiant energy of the sun, as in the sun curing of roughages. From this viewpoint it could be assumed that flue or barn-cured hay would have less vitamin D than sun-cured hay and that little or no vitamin D would be present in roughages cured in the dark or artificially dried with no sun exposure. However, this is not the case according to results obtained at this Station. Assays made on sun-cured and barn-cured hays show practically equal amounts of vitamin D in the two hays. Furthermore, appreciable amounts of vitamin D are present in hays dried without exposure to the sun, as in a dehydrating machine or natural drying in a dark place.

A few trials at other stations have been reported showing that the nutritive and antirachitic values of barn-cured hay compare favorably with that of sun-cured hay for dairy animals, as based on such criteria as rate of growth, physical condition and analyses of certain bones from slaughtered animals. However, no vitamin D contents of the hays were given.

Wylie *et al.* (8), in a trial with yearling heifers, compared the feeding value of barn-cured hay with that of sun-cured hay. The feeding periods extended through three successive winters, each trial being 150 days in length. Each animal received daily 2 lb. of grain, 10 lb. of corn silage and hay *ad libitum*. The heifers in both groups made normal growth with no marked difference in favor of either.

Moore and Thomas (4) report the results of a feeding trial with dairy calves comparing the antirachitic values of field-cured alfalfa hay, barn-dried alfalfa hay and wilted alfalfa silage. They used three groups of dairy calves, six in each group. The calves were first depleted of their body stores of vitamin D and then fed on the above roughages for a period of 6 months. From the results obtained, it was concluded that further fundamental work is necessary. The indications to date are that barn-dried hay and wilted silage will provide sufficient vitamin D for normal functions in growing calves when fed at the usual levels of roughage feeding, *i.e.*, at the rate of 2 to 3 lb. of hay, or the equivalent, per 100 lb. of body weight.

### EXPERIMENTAL

In the fall of 1946 the author started a trial with 16 dairy calves (3 days of age) to compare mainly the antirachitic value of barn-cured hay

Received for publication January 23, 1948.

<sup>1</sup> Published with the approval of the Director of the Vermont Agricultural Experiment Station.

with that of sun-cured hay up to first calving. A number of vitamin D assays were made upon the hays, bloods of living animals, and livers of slaughtered animals. At the end of 12 months the animals on barn-cured

TABLE 1  
*Vitamin D in sun-cured, barn-cured and artificially dried hays*

	Av. dry matter	Date sampled	Date assayed	USP units per g. hay		
				First sampling	Second sampling	Third sampling
<i>1946 crop cut 6/27 and 7/1</i>	(%)					
Sun-cured—used in trial	89.85					
1st sampling		6/30 and 7/4/46	8/29/46	0.61		
2nd "		1/6/47	1/24/47		0.34	
3rd "		6/19/47	6/25/47			0.32
" "		"	"			0.61
" "		"	12/2/47			0.55
" "		"	12/16/47			0.43
" " av.						0.48
Barn-cured—used in trial	89.61					
1st sampling		7/26/46	8/29/46	0.51		
2nd "		1/6/47	1/24/47		0.33	
3rd "		6/19/47	6/25/47			0.26
" "		"	"			0.18
" " av.						0.22
Artificially dried (Ardrier)	90.50					
1st sampling		6/27 and 7/1/46	12/16/46	1.14		
" "		"	5/22/47	1.40		
2nd "		3/17/47	3/27/47		0.42	
<i>1947 crop cut 7/14, 7/16 and 7/24</i>						
Sun-cured—used in trial	92.19					
1st sampling		7/16 and 7/25/47	8/13/47	2.00		
2nd "		1/14/48	1/27/48		1.80	
Barn-cured—used in trial	90.50					
1st sampling		8/7/47	8/13/47	2.33		
2nd "		1/14/48	1/27/48		2.00	
Artificially dried (Ardrier)	91.95					
1st sampling		7/14 and 7/16/47	8/28/47	0.59		
2nd "		1/14/48	1/27/48		0.75	

hay were fully equal to those on sun-cured hay in rate of growth, activity and physical appearance. This trial is still in progress and the detailed procedure and results will be reported at a later date. The present paper



deals with the vitamin D content of the various lots of hay handled in different ways. Vitamin D determinations were made according to the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII (5). The vitamin content was calculated by establishing an equation for a curve of response essentially as outlined by Coward (3). Tables 1 and 2 give the USP units of vitamin D per gram of material in the various roughages. The three hays of the 1946 crop were from the same cuttings (table 1). Two lots of hay were harvested and in each case representative samples of the three hays were obtained. Lot I was cut 9 a.m. on June 27; the hay for barn curing was hauled in at 2 p.m. June 28, and the sun-cured hay at 4 p.m. June 29. The weather was sunny except for a shower of short duration on the first day. The material for artificial

TABLE 2  
*Vitamin D in hays cured in the dark (except no. 7)*

Plot	Sample	Date cut		Dry matter	USP units
		(1947)		(%)	(per g.)
A	1	7/3	Plants cut at 5 a.m.	90.92	0.75
	2	7/3	Plants cut at 5 p.m.	91.80	0.54
B	3	8/7	Top of plants, green	91.78	0.84
	4	8/7	Bottom of plants, mostly brown	91.73	1.00
C	5	8/18	Green leaves, hand picked	91.41	0.84
	6	8/18	Brown leaves, hand picked	91.33	1.10
	7	8/18	Hay, whole plant, sun-cured	91.27	2.30

drying was obtained by following the mower and taking a handful of grass every few feet. This was placed in burlap bags so as not to allow any sun exposure. As soon as the mowing was completed, these bags of grass were taken to the barn, chopped and put through the hay drier (Ardrier). The drying involved but a few minutes. The dried material then was spread out on the floor of a dark barn loft for cooling and to complete the drying. Normally a roughage would be wilted until the moisture content was down to around 65 per cent before putting it through the drier. A few days later the material was mixed and sampled. The sample of sun-cured hay was taken from each load as it was hauled in and the barn-dried sample was taken after it had cured, which required approximately 2 weeks.

Lot II was handled in the same manner. It was cut at 4 p.m. July 1; the hay for barn curing was hauled in at 4 p.m. July 2 and the sun-cured hay at 4 p.m. July 3. A small shower occurred during the first night after cutting; otherwise the weather was sunny. The composition of a mixture of these two lots would average approximately 63 per cent timothy and grass, 27 per cent alfalfa, 7 per cent clover and 3 per cent weeds.

Composite samples were made of the two lots each of sun-cured, barn-cured and artificially dried hay. They then were milled and appropriate amounts sent to the assaying laboratory.

The 1947 crop was handled similarly. Lot I was cut at 9 a.m. July 14; the hay for barn curing was hauled in at 11 a.m. July 15 and the sun-cured hay at 2 p.m. July 16. The weather was sunny for the most part and no rain occurred. Lot II was cut at 9 a.m. July 16; the hay for barn curing was hauled in at 10 a.m. July 17. The sun-cured hay was thoroughly soaked with a shower and so it was decided to discard this hay and make another cutting from the same field to obtain sun-cured hay without rain. Another supply for barn curing and artificial drying was obtained at the same time and these samples were mixed with those from Lots I and II. This cutting was made at 9 a.m. July 24; the hay for barn curing was hauled in at 10 a.m. July 25, and since it was a fast-drying day, the sun-cured hay was ready to be hauled in at 4 p.m. on the same day.

Table 1 shows the vitamin D contents of the sun-cured and barn-cured hays used in the calf-feeding trial. Assays for the artificially dried hay harvested from the same lots are included for comparison. Three samples were taken from the 1946 crop of sun-cured and barn-cured hays—one at harvest time or soon afterward, one in January and the last one the following June. The same number will be taken from the 1947 crop. In the 1946 crop there is very little difference in the vitamin D content of the sun-cured and barn-cured hays in the first two samples taken, the units per g. being 0.61 and 0.34 for the sun-cured hay and 0.51 and 0.33 for the barn-cured hay, respectively. The June sample, however, being re-assayed several times, showed greater differences, the sun-cured hay averaging 0.48 and the barn-cured hay 0.22 unit. These results suggest that there may be a tendency for some loss of vitamin D in storage.

The hay which was dried artificially in 1946 had a decided advantage in the amount of vitamin D. The first assay showed 1.14 units per g. and a re-assay of the same sample showed 1.40 units. Another sample from the reserve supply which was stored unmilled from July, 1946, to March, 1947, contained 0.42 unit. These relatively high figures for artificially dried hay were surprising. However, Wallis (7) reported 812 units of vitamin D per pound of artificially dried hay. His sample was cut after dark and dried artificially in a dehydrating machine, thus eliminating any exposure to sunshine. Bechdel *et al.* (1) reported 150 and 300 units of vitamin D per lb. of dehydrated alfalfa hay for two successive seasons. The alfalfa was cut after sundown and dried in an artificial drier to prevent exposure to sunlight after mowing. It was planned to use this hay in a rachitogenic diet for dairy calves, but the amounts of vitamin D were found to be too large. On the other hand, Bechdel and Landsburg (2) found a measurable difference in the antirachitic potency of

dehydrated and sun-cured alfalfa hay when fed to calves as supplements to a basal rachitic diet. Two and one-half pounds of dehydrated alfalfa did not prevent the development of a mild rachitic condition over a 6-month feeding period whereas an equal amount of sun-cured alfalfa served as a complete preventive.

The first two samples of the 1947 crop show higher vitamin D contents in both the sun-cured and barn-cured hays over the previous year. The barn-cured hay was a little higher than the sun-cured hay, the former containing 2.33 and 2.00 and the latter containing 2.00 and 1.80 units. However, the artificially dried hay contained only 0.59 and 0.75 unit per g. Other samples of these hays will be assayed, but to date the barn-cured hay for both years is practically equal to the sun-cured hay in vitamin D content.

The results of the assays on these hays indicate that vitamin D may be present in the growing plant to a larger extent than heretofore has been considered to be the case. In order to throw some light upon this point, several samples of hay were cut from the same field and cured in a dark barn loft, the plants thus receiving no exposure to the sun after being cut. Samples 1 and 2 were cut at 5 a.m. and 5 p.m., respectively, to note any effect the sun might have upon the standing plant (table 2). Samples 3 and 4 were taken to show any differences in the vitamin D content of the top and bottom parts of the plants. The upper part was clipped from the lower without taking any special pains to separate the green stems and leaves from the brown. The lower part, nevertheless, consisted mainly of browned leaves and stems. Samples 5 and 6 were hand picked to obtain only green leaves and brown leaves, respectively, no stems being included. A sample of the entire plants from the same area was sun-cured for comparison. It required around 7 to 10 days for the samples to cure in the dark barn loft. They then were milled and sampled for assaying.

All the samples cured in the dark contained appreciable amounts of vitamin D. They contained lower amounts of the vitamin than did the sun-cured sample, but were higher for the most part than the sun-cured hays obtained in 1946 (table 1). Comparing samples 1 and 2, more vitamin D was present in the morning-cut sample than in the evening-cut hay, even though the standing plants of the latter received 12 hours of sunshine, the respective amounts being 0.75 and 0.54 unit per g. Samples 3 and 4 show a little less vitamin D in the upper than in the lower part of the plants, the respective amounts being 0.84 and 1.00 unit. Thus the brown part appears to contain more of the vitamin than the green part. This also is shown in a comparison of samples 5 and 6, the amounts of vitamin D in the green and brown leaves being, respectively, 0.84 and 1.10 units. The entire plant, as represented by the sun-cured sample 7, contained 2.30 units, which is more than double the amount in either sample 5 or 6 taken at the same time and cured in the dark. This shows the effect of sunshine



in increasing the vitamin D content. The amount in all probability would be higher still if only the leafy portion was considered. Wallis (6) found that the leaves of a good quality green colored alfalfa hay were about six times as potent in vitamin D as the stems. The International Units were 10.45 and 1.72 per g., respectively. Since these hays cured in the dark contained, for the most part, more vitamin D than the sun-cured hays (1946 crop) fed during the first year of the calf feeding trial, one might conclude that they also would provide sufficient amounts of vitamin D to prevent rickets.

#### CONCLUSIONS

More work needs to be done on the vitamin D content of roughages. The limited results of this study suggest that plants cured in the dark contain appreciable amounts of vitamin D. The brown leaves on growing plants appear to have a somewhat higher vitamin D content than the green leaves. Sunshine plays an important part in the formation of additional vitamin D during the curing process of roughages.

#### ACKNOWLEDGMENTS

The author is grateful to Standard Brands, Inc., New York, N. Y., for a grant which defrayed the expenses of the vitamin D assays made in connection with the calf feeding trial in which the hays included here were used, and also to Miss Grace McGuire of the Laboratory of Industrial Hygiene, Inc., New York, N. Y., under whose direction all the assays were made. Further acknowledgment is made to Mr. O. M. Camburn, Department of Animal Husbandry, University of Vermont, for providing the hays used in the calf feeding trial, to Dr. H. B. Ellenberger, Head, Animal and Dairy Husbandry Department, University of Vermont, and to Dr. C. A. Smith and Dr. G. C. Wallis of Standard Brands, Inc., for suggestions and criticisms.

#### REFERENCES

- (1) BECHDEL, S. I., HILSTON, N. W., GUERRANT, N. B., AND DUTCHER, R. A. The Vitamin D Requirement of Dairy Calves. Pa. Agr. Expt. Sta. Bull. 279. P. 9. 1932.
- (2) BECHDEL, S. I., AND LANDSBURG, K. G. The Vitamin D Requirement of Dairy Calves. Pa. Agr. Expt. Sta. Bull. 279. P. 9. 1932.
- (3) COWARD, K. H. Biological Standardization of the Vitamins. Wm. Wood and Co., Baltimore, Md. 1938.
- (4) MOORE, L. A., AND THOMAS, J. W. The Antirachitic Properties of Field-cured Hay, Barn-dried Hay, and Wilted Grass Silage when Fed to Calves. J. Dairy Sci., 30: 583-584. 1947.
- (5) U. S. PHARMACOPOEIA. XII. Rev. Mack Printing Co., Easton, Pa. 1942.
- (6) WALLIS, G. C. A Comparison of the Vitamin D Potency of the Stemmy and Leafy Portions of Alfalfa Hay. J. Agr. Research, 57: 393-396. 1938.
- (7) WALLIS, G. C. Vitamin D Deficiency in Dairy Cows. S. Dak. Agr. Expt. Sta. Bull. 372, Rev. P. 12. 1946.
- (8) WYLIE, C. E., HINTON, S. A., AND SCHALLER, J. A. Studies with Barn Air-cured Alfalfa Hay. J. Dairy Sci., 23: 555. 1940.

## THE EFFECT OF SOYA-PHOSPHATIDES ON THE ABSORPTION AND UTILIZATION OF VITAMIN A IN DAIRY ANIMALS<sup>1</sup>

G. C. ESH,<sup>2</sup> T. S. SUTTON, J. W. HIBBS, AND W. E. KRAUSS

*Department of Dairy Husbandry and the Institute of Nutrition and Food Technology,  
The Ohio State University, Columbus, and Ohio Agricultural  
Experiment Station, Wooster*

It has been shown by various research workers (2, 6, 8, 35) that the liver of the newborn calf contains either little or no vitamin A. Analyses of blood of newborn calves also revealed that the level of vitamin A is exceptionally low (19, 20, 27, 32, 35).

Colostrum, which has been found to be very rich in this vitamin (6, 16, 24, 29, 35), is the first natural material which the calves consume to overcome this deficiency. There is a wide variation in colostrum vitamin A among cows of the same breed (29) as well as in different seasons. Attempts have been made to increase the vitamin A potency of colostrum by feeding extra vitamin A during the latter part of the gestation period. Stewart and McCallum (30) failed to find an increase of this vitamin in colostrum following the feeding of carrots or cod-liver oil. Contrary to this finding, Spielman *et al.* (28) were able to demonstrate an increased amount of vitamin A in colostrum and in the blood and livers of calves following the feeding of large doses of the vitamin during the later stages of the gestation period. Wise *et al.* (35) also found higher levels of vitamin A in both the blood and the liver of the newborn calves when the dams were fed supplementary vitamin A during the gestation period. When large doses of vitamin A or carotene were fed during the later stages of gestation, the decrease in the blood plasma vitamin A and carotene of cows at parturition was not prevented (5, 17, 33, 34), although a higher level was maintained than was observed in the controls.

Numerous reports have been published regarding the importance of vitamin A in calfhood nutrition. Attempts were made to raise calves on skim milk supplemented with vitamin A concentrate (18, 19), usually with disappointing results. Wisconsin workers (19) reported that vitamin A, together with ascorbic acid and nicotinic acid, would increase the survival rate of calves on skim milk. Later work (12, 21) showed no beneficial effect of nicotinic acid feeding, especially in conjunction with large doses of vitamin A supplement.

The question is raised as to whether or not other sources of vitamin A or carotene along with skim milk can be utilized in the same way as the

Received for publication January 23, 1948.

<sup>1</sup> The data in this article are taken from a part of the dissertation presented by G. C. Esh in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University.

<sup>2</sup> India Government Research Fellow.

colostral vitamin A or carotene. Spielman *et al.* (26) have shown that the carotene of commercial concentrates is poorly utilized by the very young calf. They have reported further that the vitamin A and carotene in colostrum are used more effectively than are vitamin A and carotene added to skim milk or similarly fortified reconstituted skim milk. Knowledge of the exact mechanism of absorption and utilization of vitamin A in the animal system still is inadequate.

Considerable work has been done during recent years regarding the synergism of vitamin E and vitamin A (10, 13) as well as of lecithin and vitamin A. It has been reported that in the case of rats (25), chicks (22), and man (1), the utilization of vitamin A and carotene can be influenced by soybean phosphatides.

The purpose of the present investigation was twofold: (a) To determine the extent to which the vitamin A level of the blood and colostrum of cows and the vitamin A reserve of the newborn calves may be affected by feeding large doses of vitamin A along with soybean phosphatides during the later stages of gestation, and (b) to throw some light on the mechanism of absorption and utilization of vitamin A by calves on a skim milk ration from the time of birth.

#### EXPERIMENTAL PROCEDURE

This experiment was undertaken during the late winter and early spring months of 1947 while all the animals were on a winter herd ration. Twenty-six pregnant dairy cows of the Jersey and Holstein breeds previously maintained under similar conditions of feeding and management were divided into three dietary groups approximately 30 days prior to parturition. Group I, consisting of ten cows, was again subdivided into two groups of five each. Group I-A received only the usual herd ration<sup>3</sup> and Group I-B the same ration plus 10 g. of soybean lecithin<sup>4</sup> daily. Group II, consisting of eight cows, was given one million I.U. (International Units) of vitamin A<sup>5</sup> daily, in addition to the herd ration. Group III, consisting of eight animals, received the herd ration plus one million I.U. of vitamin A and 10 g. of lecithin daily.

These rations were continued to the seventh day following the day of parturition. The vitamin A was given orally in gelatin capsules at approximately the same hour each day. The lecithin was mixed carefully with the vitamin A concentrate (fish-liver oil) and special gelatin cap-

<sup>3</sup> The herd ration consists of ground corn 400, ground oats 300, wheat bran 100, soybean oil meal 100, salt iodized 9, and the silage and hay *ad libitum*.

<sup>4</sup> Soybean lecithin supplied by the American Lecithin Company, Long Island City, New York, contained 70 per cent soya phosphatides (lecithin, cephalin and lipositol) and 30 per cent soybean oil.

<sup>5</sup> Vitamin A capsules were supplied by the Gelatin Products Company, Detroit, Michigan. One gram of this capsule had 25,000 I.U. vitamin A.



sules each containing 3.33 g. of lecithin and 333,333 I.U. of vitamin A were prepared each week and stored in the refrigerator. Three of these capsules were administered daily to the cows in Group III.

Blood plasma vitamin A and carotene were determined each week before parturition, at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Vitamin A, carotene and lecithin analyses were made on the colostrum and milk samples successively at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Two cows in each of Groups II and III were fed in the same way for approximately 60 days before parturition in order to determine the possible effect of feeding vitamin A for a longer period on the changes of vitamin A and carotene levels in the blood plasma of the cows and their calves and in the liver of the calves as well as in the colostrum and milk.

Blood plasma vitamin A and carotene were determined in the newborn calves before they had access to colostrum or other feed. A few calves from each of the representative groups were slaughtered after birth to determine the liver storage of vitamin A and carotene. All of the calves which were not slaughtered at birth from the above experiment (15 in all) were divided into three groups. There was no predetermined basis for the allotment of the calves to the various groups except that the largest number of calves from cows fed vitamin A and lecithin were allotted to group B, which was assumed to be the group receiving the most rigorous treatment. Preliminary data had shown higher liver storage in the calves from these cows. The calves in Group A were fed colostrum at the rate of 10 lb. per 100 lb. of bodyweight for 7 days after birth. A composite mixture of colostrum was made and stored previously to standardize the feeding in every case. The vitamin A, carotene and lecithin contents of this mixture of colostrum were determined, and the total vitamin A and lecithin consumed by each calf were calculated. The calves in Group B were fed skim milk at the same rate for 7 days after birth. They were not permitted to receive any colostrum. The same quantity of vitamin A consumed daily by the calves in the colostrum-fed group (Group A) was added to the skim milk every day. The calculated quantity of vitamin A oil (25,000-37,500 I.U.) was homogenized with the skim milk before each feeding. Group C was fed skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3-4.5 g.) as was consumed daily by the calves in Group A. This feeding schedule was continued for 7 days following birth. The appropriate quantities of lecithin and vitamin A oil were homogenized with a small amount of skim milk and then mixed with the skim milk to be fed at each feeding. The surviving calves of all the groups were fed whole milk after the seventh day.

Vitamin A and carotene of blood plasma were determined successively at birth, and at 1, 3, 7, 14, and 21 days after birth. After 21 days, some

of the calves from each of the representative groups were slaughtered to determine the total liver storage of vitamin A and of carotene.

Vitamin A and carotene of blood plasma were determined according to the method of Kimble (15). The vitamin A of the blood samples having carotene concentrations exceeding 300 was determined by the method of Boyer *et al.* (3). The vitamin A and carotene of the colostrum and milk samples were determined by the method of Boyer *et al.* (4), with slight modifications. Instead of cold saponification, a hot saponification procedure was adopted. Five milliliters of colostrum or 25 ml. of milk plus 10 g. of caustic potash plus 50 ml. of methyl alcohol were refluxed in a boiling water bath for 10 minutes in a low actinic flask with a ground glass fitted reflux assembly. After cooling, the mixture was transferred to a separatory funnel, rinsing the flask with 55 ml. of water. The mixture was extracted successively with 50-ml. and with 25-ml. quantities of diethyl ether. The remainder of the procedure was the same as that of Boyer *et al.* (4). Liver vitamin A and carotene were determined by using the extraction procedure of Guilbert and Hart (8), with slight modification. Lecithin was determined according to the procedure adopted by Horrall (14). All of the colorimetric measurements were made in an Evelyn Photoelectric Colorimeter, using the appropriate filters.

#### RESULTS AND DISCUSSION

*Effect of prepartal vitamin A and lecithin feeding on the vitamin A and carotene levels in the blood plasma of cows.* The individual animal data are too voluminous to report; therefore the data are summarized in table 1. It will be noted that the feeding of lecithin or of vitamin A, or a combination of lecithin and vitamin A, did not prevent a decrease in blood plasma vitamin A and carotene at the time of parturition and beginning lactation. However, higher vitamin A levels were maintained when vitamin A was fed with or without additional lecithin (34). When both vitamin A and lecithin were fed, the highest blood vitamin A level was maintained; the level following parturition was higher (statistically significant) than that found 4 weeks prior to parturition. These data are considered as presumptive evidence that lecithin facilitates the absorption of vitamin A. There was considerable individual variation both in the time when the maximum decrease was noted after parturition and in the magnitude of the decrease. If samples had been obtained at more frequent intervals, perhaps these differences between groups would have been more clear-cut. Feeding of lecithin apparently has a tendency to delay the time of maximum postpartum decrease in blood plasma vitamin A. Although the vitamin A and lecithin feeding was discontinued on the seventh day postpartum, a carry-over effect was still apparent on the twenty-first day, the greatest carry-over effect being observed when both vitamin A and

TABLE 1  
The effect of feeding vitamin A and vitamin A plus lecithin on the average concentration of vitamin A and carotene in the blood plasma of cows for the period 4 weeks before parturition to 3 weeks following parturition

Groups	No. of cows	Days prepartum				At parturition	Days postpartum				
		28	21	14	7		1	3	7	14	21
Vitamin A in blood plasma ( $\gamma$ /100 ml.)											
I-A (No lecithin) .....	5	20.5	19.1	17.8	18.0	13.7	9.9	13.8	11.5	17.1	18.8
I-B (With lecithin) .....	5	17.9	18.8	11.9	10.1	12.4	12.4	6.7	12.1	13.7	12.4
II (Vitamin A) .....	8	19.5	29.6	25.8	26.1	20.4	18.4	21.8	26.0	23.1	22.1
III (A and lecithin) .....	8	18.9	38.9	38.9	31.2	29.9	25.4	27.5	24.7	26.8	28.7
Carotene in blood plasma ( $\gamma$ /100 ml.)											
I-A (No lecithin) .....	5	285	335	320	273	212	157	212	215	242	371
I-B (With lecithin) .....	5	303	368	340	347	318	273	303	331	297	303
II (Vitamin A) .....	8	422	351	294	222	181	160	141	145	170	263
III (A and lecithin) .....	8	390	322	240	216	159	145	128	93	133	172



lecithin were fed. These data provide additional evidence that high levels of vitamin A in the blood plasma of cows can be maintained by high levels of vitamin A feeding (34) and that this can be done more effectively by feeding a combination of vitamin A and lecithin.

TABLE 2

*The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the vitamin A content of cows' colostrum and milk up to 21 days postpartum*

Cow no.	Vitamin A of colostrum and milk ( $\gamma$ /100 ml.)					
	At parturi- tion	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	496	67	35	19	17	18
873J	145	79	21	16	15	11
820J	141	52	33	11	14	15
866J	134	69	68	22	13	14
620H	218	113	29	18	.....	.....
Mean .....	226	76	37	17	15	15
Group I-B (control group with lecithin)						
798H	143	46	.....	18	22	25
616H	132	60	58	22	19	17
801H	98	40	21	.....	.....	.....
763J	216	105	43	27	17	18
800J	134	92	22	25	26	16
Mean .....	145	69	36	23	21	19
Group II (vitamin A)						
755H	229	167	123	31	25	32
692J	420	131	179	71	23	25
812J	163	172	141	97	14	.....
711J	304	.....	169	201	74	27
649J	216	222	32	144	28	27
752H	705	302	161	191	.....	.....
Mean .....	339	197	134	122	33	28
Group III (vitamin A plus lecithin)						
852H	552	113	175	70	31	24
806H	598	156	195	77	31	39
743H	377	158	112	148	146	22
675J	847	210	125	148	25	40
760J	1119	506	165	162	29	32
Mean .....	698	229	154	121	52	32

The effect of feeding lecithin alone is not statistically significant although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

The effects of feeding vitamin A and vitamin A plus lecithin on blood plasma carotene at the time of parturition are difficult to interpret be-

cause of the depressing effect (7) of vitamin A feeding on blood plasma carotene. This depressing effect was strikingly shown in the case of three cows fed vitamin A and lecithin for 8 weeks prepartum. The average carotene content of the blood of these cows was 329, 53 and 73  $\gamma$  per 100 ml. at 8 weeks prepartum and at 7 and 21 days postpartum, respectively.

TABLE 3

*The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the carotene content of cows' colostrum and milk up to 21 days postpartum*

Cow no.	Carotene of colostrum and milk ( $\gamma$ /100 ml.)					
	At partu- rition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	195	42	18	9	14	17
873J	200	157	17	13	20	19
820J	191	74	36	18	22	28
866J	179	105	52	22	22	9
620H	133	71	18	7	.....	.....
Mean .....	180	90	28	14	19	18
Group I-B (control group with lecithin)						
798H	223	53	.....	11	10	14
616H	200	89	46	16	35	8
801H	105	34	17	.....	.....	.....
763J	218	216	53	16	14	13
800J	172	58	10	8	12	9
Mean .....	183	90	31	13	18	11
Group II (vitamin A)						
755H	140	108	48	12	8	8
692J	137	36	20	8	8	15
812J	113	62	18	10	7	.....
711J	152	.....	34	27	8	9
649J	103	69	10	18	7	28
752J	115	47	10	6	.....	.....
Mean .....	126	64	23	14	8	15
Group III (vitamin A plus lecithin)						
852H	171	24	15	6	6	6
806H	195	27	25	9	6	12
743H	168	30	16	6	17	7
675J	243	71	26	6	7	15
760J	200	42	16	6	6	6
Mean .....	195	39	20	7	8	9

*Effect of prepartal vitamin A and lecithin feeding on the vitamin A, carotene and lecithin content of colostrum.* The data in table 2 show that when vitamin A was fed, the vitamin A in the colostrum was significantly higher than that produced by the groups receiving no vitamin A. When

both vitamin A and lecithin were fed, the vitamin A in the first milking of colostrum was approximately double that of the colostrum from cows receiving vitamin A alone. This difference became less as the milk approached normal and had vanished by the seventh day postpartum. The effects of vitamin A feeding on the potency of the milk still were evident 2 weeks following the end of the vitamin feeding.

The data on the effect of feeding lecithin and vitamin A on the carotene content of the colostrum and early milk are presented in table 3. The feeding of vitamin A depresses the level of carotene in colostrum and milk, a result which has been reported previously (7). It appears also that the feeding of lecithin with the vitamin A enhances this suppressing effect. Additional data are needed to confirm this point.

As previously noted, three animals were fed vitamin A and lecithin for a period of 8 weeks before freshening. The limited data obtained from these animals indicated that higher levels of vitamin A were maintained in the milk after the third day following parturition and the milk carotene was further depressed. These limited data need further confirmation.

The carotene content of colostrum and milk follows the same trend as in the blood. The apparent antagonistic effect of supplemental vitamin A on blood and milk carotene is not explainable in the light of present knowledge; however, its occurrence seems to be beyond doubt.

The total output of vitamin A in International Units per milking is presented in table 4. Although the level of carotene has been depressed in the vitamin A supplemented groups, the total output of vitamin A is higher in these groups. When lecithin was fed with vitamin A, the total output of vitamin A was highest, especially at the first milking. This difference is so great that there is little doubt of its significance. Following the discontinuation of vitamin A supplementation on the seventh day, a sudden marked drop in vitamin A occurred. In the case of those animals receiving both vitamin A and lecithin, the drop was more gradual. Results in the control groups are interesting. Although the vitamin A concentration in the milk of the control group I-A (without lecithin) was higher than that of the control group I-B (with lecithin, see table 2), the total output of vitamin A is higher in the lecithin-fed group. This is due to the greater milk yield. Whether this increased milk-yield is due to lecithin feeding is to be determined by further experiments with a larger number of cows.

The effects of lecithin feeding on the lecithin content of colostrum and milk are shown in table 5. The lecithin content of colostrum and milk seems to be maintained at a higher level when both vitamin A and lecithin are fed. The carry-over effect is still apparent in the milk on the twenty-first day, 2 weeks after the supplemental feeding was discontinued. When



lecithin is fed without vitamin A or vitamin A fed without lecithin, the amount of lecithin in the colostrum and milk is little different from that of the control group which received no supplement. It appears from these data that there may be a reciprocal relationship in the absorption and/or metabolism of these two compounds.

TABLE 4

*The effects of feeding vitamin A and vitamin A plus lecithin on the changes in total output of vitamin A per milking up to 21 days postpartum*

Cow no.	I.U. <sup>a</sup> of vitamin A per milking					
	At parturi- tion	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	136,140	13,557	13,648	6,717	7,541	8,323
873J	16,565	11,831	3,696	3,769	4,482	3,601
820J	17,998	15,731	8,759	4,839	5,723	6,937
866J	9,456	8,361	8,480	5,133	4,732	3,932
620H	50,592	23,273	6,445	4,147	.....	.....
Mean .....	46,150	14,550	8,206	4,921	5,620	5,698
Group I-B (control group with lecithin)						
798H	55,600	34,610	.....	9,899	13,075	17,668
616H	51,950	37,710	25,264	11,195	13,078	8,400
801H	87,780	19,854	9,351	.....	.....	.....
763J	72,350	5,304	19,440	8,629	6,353	7,770
800J	20,512	30,058	5,994	8,146	8,781	3,964
Mean .....	57,638	25,507	15,012	9,467	10,321	8,950
Group II (vitamin A fed)						
755H	58,883	90,360	47,782	16,685	12,693	16,993
692J	112,493	41,939	60,538	25,285	7,645	12,844
812J	45,740	37,675	45,764	32,017	6,355	.....
711J	36,652	.....	52,145	67,821	21,279	8,831
649J	34,285	24,123	6,137	41,377	8,436	13,443
752H	280,100	125,412	60,156	72,996	.....	.....
Mean .....	94,692	63,902	45,420	42,697	11,282	13,027
Group III (vitamin A plus lecithin)						
852H	102,850	22,056	55,220	19,315	9,649	6,955
806H	455,910	51,539	112,227	33,145	14,169	23,099
743H	295,940	66,164	35,697	59,653	76,830	10,979
675J	146,240	55,198	30,774	42,357	9,092	15,751
760J	141,713	56,997	45,183	51,164	9,417	9,253
Mean .....	228,530	50,391	55,820	41,127	23,830	13,207

<sup>a</sup> One microgram of vitamin A = 4 I.U. Vitamin A. One microgram of carotene = 1.66 I.U. vitamin A.

*Effect of supplementing the maternal diet with vitamin A and lecithin on the blood plasma vitamin A and carotene of the newborn calf.* The results of this phase of the study are presented in table 6. The plasma vita-

min A of the calves from cows receiving vitamin A was significantly higher than that of those from the control cows. These results are in agreement with those of Wise *et al.* (35) and Spielman *et al.* (27). The

TABLE 5

*The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the lecithin content of cows' colostrum and milk*

Cow no.	Percentage of lecithin in colostrum and milk					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	0.062	0.051	0.049	0.028	0.029	0.024
873J	0.076	.....	0.044	0.039	0.038	0.038
820J	0.058	0.059	0.043	0.038	0.021	0.020
866J	0.068	.....	0.049	0.039	0.029	0.036
620H	0.060	0.080	0.048	0.038	0.034	.....
Mean .....	0.065	0.063	0.047	0.036	0.030	0.029
Group I-B (control group with lecithin)						
798H	0.076	0.056	.....	.....	0.042	0.026
616H	0.076	.....	0.056	.....	0.021	0.025
801H	0.051	0.043	0.023	.....	0.042	0.026
763J	0.073	.....	0.074	.....	.....	.....
800J	0.055	0.053	0.055	0.048	0.026	0.029
Mean .....	0.066	0.051	0.052	.....	0.033	0.026
Group II (vitamin A)						
755H	0.068	0.063	0.055	0.046	0.031	0.025
692J	0.064	0.046	0.052	0.047	0.024	0.023
812J	0.056	0.046	0.044	0.039	0.036	.....
711J	0.088	.....	0.074	0.055	0.036	0.040
649J	0.064	0.035	0.026	0.060	0.036	0.038
752J	0.084	0.067	0.059	0.052	.....	.....
Mean .....	0.071	0.051	0.052	0.049	0.033	0.032
Group III (vitamin A plus lecithin)						
852H	0.094	0.062	0.057	0.058	0.044	0.036
806H	0.094	0.060	0.050	0.051	0.046	0.051
743H	0.069	0.049	0.040	0.040	0.046	0.030
675J	0.093	0.100	0.180	0.081	0.059	0.055
760J	0.093	0.102	0.075	0.056	0.052	0.051
Mean .....	0.089	0.075	0.060	0.057	0.049	0.045

slightly higher mean value of the blood plasma vitamin A of calves from cows receiving both vitamin A and lecithin is insignificant.

It appears from the data on blood plasma carotene that a combination of lecithin and vitamin A in the maternal diet exerts a suppressing action on the level of carotene in the blood of the newborn calf. These data, however, are within a range where experimental error of determination is

apt to be rather high, and the difference may not be as significant as the statistic indicates.

*The effect of supplementing the maternal diet with vitamin A and lecithin on the liver storage of vitamin A and carotene in the newborn calf.* Seven of the calves were sacrificed at birth to determine total liver storage

TABLE 6

*The effect of increasing the vitamin A and lecithin content of the maternal diet on the blood plasma and liver storage of vitamin A and carotene in the newborn calf*

Dam no.	Calf no.	Blood plasma data		Liver storage data		
		Vitamin A	Carotene	Liver wt.	Vitamin A	Total A
		( $\gamma$ /100 ml.)	( $\gamma$ /100 ml.)	(g.)	( $\gamma$ /g.)	( $\gamma$ )
Calves from Group I (control cows)						
866J	819J	2.4	4.2	.....	.....	.....
873J	820J	3.5	6.3	.....	.....	.....
851H	825H	6.5	11.4	.....	.....	.....
800J <sup>a</sup>	944J	7.7	4.2	.....	.....	.....
820J	821J	5.4	9.2	434	0.59	249
620H	948H	8.9	0.0	1000	0.25	252
801H <sup>a</sup>	828H	8.4	5.6	793	0.08	67
	Mean .....	6.1	5.8	.....	0.31	190
Calves from Group II (cows fed vitamin A)						
755H	905H	7.2	11.4	.....	.....	.....
812J	946J	9.8	8.5	.....	.....	.....
711J	823J	8.6	8.4	.....	.....	.....
853H	830H	9.3	0.0	.....	.....	.....
752H	829H	10.7	0.0	.....	.....	.....
692J	822J	11.5	4.9	435	12.0	5241
649J	827J	6.2	7.0	500	13.1	6580
	Mean .....	9.0	5.7	.....	12.5	5910
Calves from Group III (cows fed vitamin A plus lecithin)						
855J	832J	9.2	0.0	.....	.....	.....
760J	947J	6.6	2.8	.....	.....	.....
743H	950H	10.7	0.0	.....	.....	.....
852H	949H	10.2	0.0	.....	.....	.....
857H	951H	13.6	0.0	.....	.....	.....
675J	824J	8.2	5.7	438	22.0	9625
806H	826H	15.1	0.0	945	14.6	13820
	Mean .....	10.5	1.2	.....	18.3	11722

<sup>a</sup> These cows were in Group I-B; lecithin was fed.

of vitamin A and carotene. Three of these calves were from cows in Group I, two from cows in Group II, and two from cows in Group III. As previously reported by other workers (27, 35), a statistically significant greater liver storage was found in newborn calves from dams receiving massive vitamin A supplements. The cows receiving both vitamin A and



lecithin gave birth to calves with almost double the liver vitamin A storage of those from cows receiving vitamin A alone. As can be noted from table 6, this difference was due in part to higher concentration and in part to greater liver weight. Although the numbers are limited, the magnitude of the difference is so great that there is little doubt of the significance, particularly in the light of the other data presented in this paper. Fur-

TABLE 7

*The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A and lecithin on the plasma vitamin A levels of calves*

Calf no.	Dam no.	Dam group	Vitamin A fed daily (I.U.)	Vitamin A in plasma (%/100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	7.2	4.6	27.9	16.9	9.4	9.3
819J	866J	I-A	25,000	2.4	8.2	24.0	9.9	13.6	8.3
820J	873J	I-A	25,000	3.5	11.9	25.4	23.3	13.5	11.1
944J	800J	I-B	25,000	7.7	13.5	18.2	19.9	14.6	14.1
832J	855J	III	25,000	9.2	11.9	20.2	20.7	18.2	15.9
			Mean	6.2	10.1	23.5	18.2	13.8	11.7
Group B (skim milk plus vitamin A)									
825H	851H	I-A	37,500	6.5	5.3	12.0	Fell sick and died on the 7th day		
946J	812J	II	25,000	9.8	6.6	.....	"	"	"
947J <sup>a</sup>	760J	III	25,000	6.6	6.6	8.7	13.9	11.3	6.6
950H <sup>a</sup>	743H	III	37,500	10.7	10.4	12.2	10.1	9.3	22.0
831J <sup>b</sup>	854J	III	25,000	5.9	9.3	13.2	13.3	died on the 11th day	
			Mean	7.9	7.6	11.4	12.4	.....	.....
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.6	15.7	15.7	18.7	16.8	13.9
949H	852H	III	37,500	10.2	10.7	30.3	35.0	20.8	16.0
951H	857H	III	37,500	13.6	10.7	19.2	27.8	17.2	10.5
830H	853H	II	37,500	9.3	18.3	14.3	28.1	12.2	15.3
829H	752H	II	37,500	10.7	13.6	16.8	22.8	12.7	12.2
			Mean	10.5	13.8	19.3	26.3	15.9	13.6

<sup>a</sup> Given 2% coconut oil per feeding.

<sup>b</sup> Given lecithin from the 6th day.

ther evidence of the effect of vitamin A feeding on liver storage is shown in table 9. Calves 832J, 831J, and 830H were from dams that received the vitamin A supplement for 8 weeks prior to parturition. When these calves were sacrificed at 21 days of age (calf 831J died at 11 days of age), the liver storage was significantly higher than that of other comparable calves with similar histories and treatment.

*Effect of the diet on the blood plasma vitamin A and carotene levels and the liver vitamin A storage of young calves.* As previously indicated, the calves from the cows in this experiment were removed from the dams at birth (before nursing) and given special dietary treatments for the first 7 days. These dietary treatments have been described earlier in this paper.

The data obtained are presented in tables 7 and 8. The feeding of colos-

TABLE 8

*The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A and lecithin on the plasma carotene levels of calves*

Calf no.	Dam no.	Dam group	Vitamin A fed daily (I.U.)	Carotene in plasma ( $\gamma$ /100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	11.4	5.7	24.7	38.8	20.6	23.2
819J	866J	I-A	25,000	4.2	6.4	4.9	62.1	49.4	37.2
820J	873J	I-A	25,000	6.3	2.8	26.3	58.7	29.0	21.8
944J	800J	I-B	25,000	4.2	21.7	48.1	22.4	51.0	35.6
832J	855J	III	25,000	0.0	5.7	29.4	35.6	57.0	32.4
			Mean	5.2	8.4	26.6	43.5	41.3	30.0
Group B (skim milk plus vitamin A)									
825H	851H	I-A	37,500	11.4	7.8	13.6	died	on the	7th day
946J	812J	II	25,000	8.5	5.6	.....	"	"	"
947J <sup>a</sup>	769J	III	25,000	2.8	5.6	7.7	0.0	12.8	5.9
950H <sup>a</sup>	743H	III	37,500	0.0	0.0	3.5	4.9	17.2	21.8
831J <sup>b</sup>	854J	III	25,000	0.0	0.0	0.0	3.5	died on the	11th day
			Mean	4.5	4.0	6.2	2.8	.....	.....
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.4	15.3	15.3	2.5	15.3	27.9
949H	852H	III	37,500	0.0	0.0	0.0	4.9	32.4	30.2
951H	857H	III	37,500	0.0	0.0	0.0	4.9	9.6	26.3
830H	853H	II	37,500	0.0	2.6	0.0	2.8	26.3	14.3
829H	752H	II	37,500	0.0	0.0	0.0	3.5	12.8	20.9
			Mean	1.7	3.5	3.1	3.7	19.3	24.0

<sup>a</sup> Given 2% coconut oil per feeding.

<sup>b</sup> Given lecithin from the 6th day.

trum resulted in a marked increase in the amount of vitamin A in the blood, as has been noted by others (19, 20, 32). There was an increase in blood plasma carotene following the feeding of colostrum (table 8), but the carotene remained low in both the groups fed vitamin A, as was expected. The carotene increase in the latter groups on the fourteenth and twenty-first days resulted from whole milk feeding following 7 days of age. There was no evidence of scours in the colostrum-fed group. The calves receiving skim milk plus the vitamin A supplement did very poorly.

Serious scours developed on the third day. Three of the calves died, two on the seventh day and one on the eleventh day. The calf that died on the eleventh day was from a cow that received both vitamin A and lecithin, and at the time of death this calf had an appreciable liver storage of vitamin A (see calf 831J, table 9). Perhaps the liver storage of vitamin A permitted this calf to endure the rigors of the diet longer than those which died on the seventh day. The other two calves, 947J and 950H, were given 2 per cent coconut oil and were able to survive. These calves also scoured from the third to the tenth day, and vitamin A absorption, as indicated by the low blood level, was poor.

TABLE 9

*The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A plus lecithin on the storage of vitamin A in the liver of calves at 21 days of age*

Calf no.	Dam no.	Dam group	Wt. of the liver (g.)	Vitamin A (γ/g.)	Total vitamin A (γ)
Calves from Group A (colostrum-fed)					
819J	866J	I-A	519.6	7.2	3,724.0
820J	873J	I-A	526.8	8.7	4,630.0
832J <sup>a</sup>	855J	III	608.0	53.0	32,244.0
Calves from Group B (skim milk plus vitamin A)					
825H <sup>b</sup>	851H	I-A	988.0	0.15	149.0
946J <sup>b</sup>	812J	II	466.5	11.8	5,524.0
831J <sup>c</sup>	854J	III	453.8	56.3	25,570.0
Calves from Group C (skim milk plus vitamin A plus lecithin)					
823J	711J	II	549.0	35.4	19,470.0
830H <sup>a</sup>	853H	II	1124.0	69.0	77,556.0
829H	752H	II	953.0	36.6	34,900.0

<sup>a</sup> The dams got vitamin A or vitamin A plus lecithin for 8 weeks prepartum.

<sup>b</sup> Died on the 7th day.

<sup>c</sup> Died on the 11th day.

The low level of blood vitamin A in the group receiving skim milk plus vitamin A indicates poor vitamin A absorption on this type of diet. When lecithin was added along with the vitamin A, the blood levels were comparable to those of the colostrum-fed group. These results again indicate that soya lecithin enhances the absorption of vitamin A. There were a few mild cases of scours among the calves in this group, but, in general, they did quite well and were comparable to the colostrum-fed calves in rate of growth and general appearance.

It has been suggested that colostrum vitamin A may be superior in the nutrition of newborn calves to the vitamin A of fish-liver oil or other concentrated sources (9). The data presented herein provide evidence that the higher concentration of lecithin present in colostrum may be partially responsible for the better absorption and utilization of colostrum vitamin A.



An attempt was made to start calves on a skim milk ration plus coconut oil (two calves) and a skim milk ration plus lecithin (two calves). These attempts failed and all the calves died on the fourth day following birth.

The results of this phase of the investigation show that unless adequate quantities of vitamin A or its precursor are present in the ration and unless favorable circumstances for vitamin A absorption are provided, the animal will quickly succumb to vitamin A deficiency, even when there is considerable liver storage.

Limited data on the liver storage of vitamin A determined on the twenty-first day are presented in table 9. Here again it will be noted that the calves fed the vitamin A plus lecithin had appreciably higher liver storages. While the data cannot provide conclusive proof that vitamin A storage is greater when vitamin A and lecithin are fed, rat data (31) have proved this point conclusively.

Previous workers (12) have shown that the feeding of vitamin A in capsules resulted in increased liver storage. The results of the present investigation provide evidence that the storage will be increased still further if lecithin is fed along with vitamin A.

#### SUMMARY

Twenty-six healthy pregnant dairy cows of the Jersey and the Holstein breeds were divided into three dietary groups approximately 30 days prior to parturition. Each group received the basic herd ration. Group I, consisting of ten cows, was again subdivided into two groups; Group I-A received no supplement and Group I-B the herd ration plus 10 g. of soya-lecithin daily. Each of the eight cows in Group II was given one million I.U. of vitamin A (fish-liver oil) daily. Each of the eight cows in Group III was fed one million I.U. of vitamin A and 10 g. of lecithin daily. The supplements were continued up to the seventh day following parturition. Assays of blood vitamin A and carotene and of milk vitamin A, carotene and lecithin were made at intervals up to 21 days postpartum. Blood plasma vitamin A and carotene were determined in all calves, and representative animals were sacrificed at birth to determine vitamin A liver storage.

At parturition the plasma vitamin A level in the control cows fell almost to half of the 4 weeks prepartum level. The level in the cows fed vitamin A supplements remained fairly high, especially for the cows fed lecithin plus vitamin A, indicating that lecithin enhanced the absorption of vitamin A. There was no significant effect of feeding lecithin without vitamin A, although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

Blood plasma carotene was depressed in both vitamin A supplemented

groups. However, when vitamin A was fed for a longer period, the carotene level was depressed still further in the cows fed lecithin along with vitamin A. These limited data indicate that lecithin enhanced the action of vitamin A in depressing the carotene level.

The vitamin A in the colostrum of cows fed vitamin A was greater than that of the control group; when both lecithin and vitamin A were fed, the colostrum vitamin A at the first milking was approximately double that of the vitamin A supplemented cows. This shows that lecithin, when added to vitamin A, increased the transmission of colostrum vitamin A. The transmission of vitamin A and carotene in milk closely followed the trend found in the blood plasma.

The lecithin content of milk was highest when lecithin was fed to the cows along with vitamin A, and a higher level was maintained in the normal milk. Feeding lecithin without vitamin A had no effect on the transfer of lecithin to milk. When both vitamin A and lecithin were fed, the lecithin of the colostrum and milk was increased.

The blood plasma vitamin A level in the newborn calf was highest and the plasma carotene level was the lowest in the calves from dams fed both lecithin and vitamin A.

The total liver storage vitamin A in the newborn calves from the control group was low (190  $\gamma$ ); it was 5,910  $\gamma$  in the vitamin A supplemented group and 11,722  $\gamma$  in the vitamin A plus lecithin supplemented group. Thus, the addition of lecithin to the vitamin A supplement remarkably increased the liver storage.

Three groups of five calves each were fed from birth to 7 days of age as follows: Group A, colostrum; Group B, skim milk plus the same daily quantity of total vitamin A consumed by the calves in the colostrum group (25,000-37,500 I.U. of vitamin A); and Group C, skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3-4.5 g.) available in the colostrum given to Group A.

Every calf in Group B developed serious scours from the third day. Two of them died on the seventh day and a third one on the eleventh day. Their blood plasma vitamin A level was much below that of the colostrum-fed calves. All the calves in Groups A and C grew quite well with slight evidence of digestive disturbance. Blood plasma levels in Group A and C were almost identical, showing the ability of lecithin to increase absorption and utilization of vitamin A.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Dr. W. D. Poun- den, of the Ohio Agricultural Experiment Station, who so generously aided in conducting the experiment, and the helpful suggestion of Dr. D. V. Josephson of The Ohio State University Department of Dairy Technol-

ogy. The authors are indebted to D. R. Whitney and his associates of The Ohio State University Statistics Laboratory for the statistical treatment of the data.

## REFERENCES

- (1) ADLERSBERG, D., AND SOBOTKA, H. Influence of Lecithin Feeding on Fat and Vitamin A Absorption in Man. *J. Nutrition*, 25: 255-263. 1943.
- (2) BARON, N. S. Vitamin A and Its Relationship to Some of the Commoner Diseases of Farm Animals. *Vet. Record*, 54: 31-35. 1942.
- (3) BOYER, P. D., PHILLIPS, P. H., AND SMITH, J. K. The Separation of Carotene from Vitamin A in Blood Plasma. *J. Biol. Chem.*, 152: 445-452. 1944.
- (4) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk—A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, 10: 101-102. 1944.
- (5) BRAUN, W. Studies on the Vitamin A and Carotene Levels in Cattle. *J. Nutrition*, 29: 61-71. 1945.
- (6) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. *Biochem. J.*, 27: 1998-2005. 1933.
- (7) DEUEL, H. J., JR., HALLMAN, L. F., JOHNSTON, C., AND MATTSO, F. The Effect of a High Vitamin A Intake on the Blood and Milk Carotene of Holstein and Guernsey Cows. *J. Nutrition*, 23: 567-579. 1942.
- (8) GUILBERT, H. R., AND HART, G. H. Storage of Vitamin A in Cattle. *J. Nutrition*, 8: 25-44. 1934.
- (9) HANSEN, R. G., PHILLIPS, P. H., AND RUPEL, I. W. The Effect of Vitamin Supplements upon Survival of New Born Calves. *J. Dairy Sci.*, 29: 761-766. 1946.
- (10) HARRIS, P. L., SWANSON, W. J., AND HICKMAN, K. C. D. Covitamin Studies. VI. Effect of Tocopherol Supplementation on the Output of Vitamin A, Carotene and Fat by Dairy Cows. *J. Nutrition*, 33: 411-428. 1947.
- (11) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage of Calves. *J. Dairy Sci.*, 29: 519-520. 1946.
- (12) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Supplementary Vitamins on Blood Composition Liver Storage and Incidence of Scours in Calves. *J. Dairy Sci.*, 30: 115-120. 1947.
- (13) HICKMAN, K. C. D., KALEY, M. W., AND HARRIS, P. L. Covitamin Studies. I. The Sparing Action of Natural Tocopherol Concentrates on Vitamin A. *J. Biol. Chem.*, 152: 303-311. 1944.
- (14) HORRALL, B. E. A Study of the Lecithin Content of Milk and Its Products. *Ind. Agr. Expt. Sta. Bull.* 401. March, 1935.
- (15) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, 24: 1055-1065. 1939.
- (16) KRAMER, M. M., BAIR, M. D., KUNERTH, B. L., AND RIDDELL, W. H. The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single Feeding Method. *J. Agr. Research*, 56: 227-232. 1938.
- (17) KUHLMAN, A. H., AND GALLUP, W. D. Changes in Blood Plasma Carotene Associated with Parturition and Lactation of Jersey Cows. *J. Dairy Sci.*, 27: 633-634. 1944.
- (18) LIERSCHER, W. Feeding Tests of Calves with Skim Milk and Codliver Oil Emulsion as a Substitute for Milk. *Tierernähr.*, 2: 11-16. 1930.
- (19) LUNQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. *J. Dairy Sci.*, 26: 1023-1030. 1943.



- (20) MOORE, L. A., AND BERRY, M. H. Effect of Colostrum on the Vitamin A and Carotene Content of New Born Calves. *J. Dairy Sci.*, 27: 867-873. 1944.
- (21) NEVENS, W. B., AND KENDALL, K. A. The Value of Supplementary Vitamin Feeding in the Rearing of Dairy Calves. *J. Dairy Sci.*, 30: 175-181. 1947.
- (22) PATRICK, H., AND MORGAN, C. L. An Unidentified Nutrient Requirement for Utilization of D1 Tocopherol by the Chick. *Science*, 98: 434-435. 1943.
- (23) RUBIN, M., AND BIRD, H. R. The Apparent Antagonism between Vitamin A and Carotinoids in the Fowl. *Science*, 103: 584-586. 1946.
- (24) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat Soluble Vitamins. XLI. The Carotene and Vitamin A of Colostrum. *J. Biol. Chem.*, 107: 697-703. 1934.
- (25) SLANETZ, C. A., AND SCHARF, A. The Effects of Phosphatides on Utilization of Vitamin A and Carotene. *Proc. Soc. Exptl. Biol. Med.*, 53: 17-19. 1943.
- (26) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. Carotene Utilization in the Newborn Calf. *J. Dairy Sci.*, 29: 381-391. 1946.
- (27) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine. *J. Dairy Sci.*, 29: 707-715. 1946.
- (28) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (29) STEWART, J., AND MCCALLUM, J. W. Vitamin A Content of the Colostrum of Dairy Cows. *J. Agr. Sci.*, 28: 428-436. 1938.
- (30) STEWART, J., AND MCCALLUM, J. W. The Effect of a Vitamin A Rich Diet on the Vitamin A Content of the Colostrum of Dairy Cows. *J. Dairy Research*, 13: 1-4. 1942.
- (31) SUTTON, T. S., AND ESH, G. C. Unpublished data.
- (32) SUTTON, T. S., AND KAESER, H. E. Some Physiological Effects of Extending the Colostrum Feeding Period of Dairy Calves. *J. Dairy Sci.*, 29: 13-26. 1946.
- (33) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A. Changes in the Level of Vitamin A and Carotene in the Blood Plasma of Dairy Cows Associated with Parturition and Beginning Lactation. *J. Dairy Sci.*, 28: 933-939. 1945.
- (34) WISE, G. H., ATKESON, F. W., CALDWELL, M. J., PARRISH, D. B., AND HUGHES, J. S. Effects of High Vitamin A Intakes on Milk and Fat Yields and on Vitamin A Contents in Milk, Blood and Livers of Dairy Cows. *J. Dairy Sci.*, 30: 279-291. 1947.
- (35) WISE, G. H., CALDWELL, M. J., AND HUGHES, J. S. The Effect of the Prepartum Diet of the Cow on the Vitamin A Reserve of Her New Born Offspring. *Science*, 103: 616-618. 1946.

## CAROTENE AND VITAMIN A IN THE COLOSTRUM OF COWS OF TYPICAL INDIAN BREEDS

B. C. RAY SARKAR<sup>1</sup>

*Animal Nutrition Section, Indian Veterinary Research Institute,  
Izatnagar, U. P., India*

Since the discovery of vitamin A as an anti-infective factor, many workers (3, 8, 14, 15, 18, 21, 24, 27) have studied its importance in the nutrition of dairy calves. It is now recognized that vitamin A is indispensable and must be supplied in adequate amounts during the earlier part of life, since the calf is born with practically no reserve of vitamin A (2, 4, 10, 17). Krauss *et al.* (17) reported a decrease in the incidence of pneumonia in calves which received 15,000 I.U. of vitamin A concentrate daily. Gullickson and Fitch (11), in an experiment involving 72 calves, reported less trouble from digestive disturbances in young calves that were fed cod-liver oil than in calves not given the vitamin A supplement. Phillips *et al.* (24) observed that the administration of shark-liver oil with a high vitamin A potency and certain members of the B-complex eliminated diarrhea and lowered the mortality resulting from pneumonia. Nelson *et al.* (23) recommended the feeding of fish-liver oil as a vitamin A supplement when there was difficulty in raising calves.

Under natural feeding conditions vitamin A supplementation is not usually practiced; however, the value of colostrum as a source of vitamin A for newborn calves has been the subject of investigation by some workers. Stewart and McCallum (30) made an extensive study of the correlation between the incidence of white scours in calves and the vitamin A content of the colostrum. In 83 calves which received colostrum containing more than 250 blue units of vitamin A, only 10.8 per cent developed white scours or allied infections; whereas, in 28 calves which received colostrum containing less than 250 blue units of vitamin A, 25 per cent developed white scours or allied infections. Moore and Berry (22) also have pointed out the significance of adequate colostrum feeding in building up the vitamin A reserve in the calf. Apart from these observations, several papers report that cow colostrum contains more vitamin A than the milk (7, 9, 12, 16, 20). Dann (5) and Kramer *et al.* (16) have shown that cows' colostrum are ten to one-hundred times richer in vitamin A activity than the normal milk. On the first day of life a calf is supposed to receive a supply of vitamin A greater than the later milk can give in 20 to 50 days. Henry *et al.* (13) have noted that the colostrum of first-

Received for publication January 26, 1948.

<sup>1</sup> Present address, Visiting Professor in the Department of Agricultural Chemistry, Michigan State College, East Lansing.

calf heifers is richer in vitamin A than that of cows, although Stewart and McCallum (29) did not note such a difference. However, they did report: (a) The length of the dry period between successive calvings affected the colostrum vitamin A. (b) The vitamin A content was independent of season in contrast to that of carotene. The vitamin A content of the colostrum collected from 100 cows varied from 35 to 1,181 I.U. per 100 ml. By the third or fourth day the amount of vitamin A was from one-tenth to one-twentieth of that present immediately after parturition.

The variations in the carotene and vitamin A content of colostrum fat from various breeds of cattle have been studied by Gillam *et al.* (9) and Semb *et al.* (26). These investigators have shown that the concentration of carotene and vitamin A in colostrum fat is from five to fifteen times that of the fat prepared from normal milk and that these constituents decrease very rapidly during the first week postpartum. Stewart and McCallum (31) were unable to raise the vitamin A content of colostrum of cows on winter feed by feeding 3 lb. of carrots or one-seventh pint of cod-liver oil per day. Spielman *et al.* (28) have studied the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum. Colostrum from cows receiving a low carotene ration for 60 days before parturition contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving a comparatively rich carotene ration. The effect of feed was more pronounced on the carotene content of the butterfat of the colostrum than on the vitamin A content, although vitamin A supplementation for 60 days before calving increased the colostrum vitamin A to a considerable extent.

As no comparable data are available for any of the milking breeds of cows in India, it seemed desirable to initiate a study along this line. The results obtained from such a study are presented in this paper.

#### EXPERIMENTAL PROCEDURE

The colostrum and milk samples were collected from 15 cows in the Institute dairy herd for a period of 8 days postpartum. Nine cows and one first-calf heifer of the Haryana breed and five first-calf heifers of the Sahiwal breed were used. The animals were fed 3.5 lb. of a dairy mixture, 1 oz. of iodized salt and 1 oz. of bonemeal per head daily. The nature of the roughage fed to the cows depended on the season of the year and has been discussed in a previous paper (25).

The colostrum and milk samples were collected each day for 8 days and stored in a refrigerator for subsequent analysis. The percentage composition with respect to fat, solids-not-fat, protein and ash was determined according to the methods outlined in the A.O.A.C. (1). The extraction procedure of Dann (5) was followed for the determination of carotene and



vitamin A. Vitamin A was measured spectrographically in an alcoholic solution of the unsaponifiable matter, and the proper correction for the absorption due to carotene was made. For the conversion of corrected density readings to micrograms of vitamin A, the factor,  $E_{1\%}^{1\text{cm.}} 328 \text{ m}\mu = 1800$ , was used. Carotene was estimated colorimetrically in a petroleum ether solution.

Table 1 gives the data pertaining to the history and breed of the animals used in this experiment.

TABLE 1  
*Data pertaining to the history of the cows*

Animal no.	No. of lactation	Length of dry period	Calving date
Hariana breed			
		(days)	
1	10	166	8-22-42
2	3	180	10-17-42
3	3	76	10-25-42
4	10	144	11-14-42
5	2	146	11-15-42
6	2	149	11-18-42
7	9	287	12-28-42
8	2	148	1-20-43
9	2	37	5-16-43
10	1	.....	5-17-43
Sahiwal breed			
11	1	.....	10-26-42
12	1	.....	1- 3-43
13	1	.....	1- 7-43
14	1	.....	1-17-43
15	1	.....	5-17-43

#### RESULTS

*Composition of colostrum milk.* The data on the average daily milk yield and the percentage composition of the colostrum milk with respect to fat, solids-not-fat, protein and ash are presented in table 2. Individual variations are quite apparent. The comparatively lower yields of colostrum and the higher percentages of the above constituents were found mostly in the samples obtained from the Sahiwal heifers, which were considered at one time as being sterile. All of the colostrum samples were characterized by a high percentage of solids-not-fat, protein and ash. The fat content of the colostrum milk from individual cows varied widely from day to day but the percentage of fat in the first two days' samples was lower than in the later milk. The change from colostrum to milk was a gradual one, a fact which has been established by others (6) but, on the whole, the colostrum samples tended to approach normal milk after the fourth day. The protein content was much higher than that usually obtained for the

TABLE 2  
Average daily yield and the percentage composition of colostrum

Days after parturition	Yield		Fat		S-N-F		Protein		Ash	
	Range	Av. <sup>a</sup>	Range	Av. <sup>a</sup>	Range	Av. <sup>b</sup>	Range	Av. <sup>b</sup>	Range	Av. <sup>b</sup>
	(lb.)	(lb.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	3-13	6.4	1.6- 8.3	4.37	12.94-24.85	18.37	8.40-18.26	13.16	0.865-1.253	1.028
2	2-14	8.3	0.9- 7.4	4.23	11.16-16.50	14.07	8.55-11.57	9.00	0.717-0.965	0.877
3	4-18	11.2	1.1- 8.1	5.25	9.33-11.9	10.35	5.46-8.26	6.30	0.717-1.043	0.858
4	2-18	11.3	4.3-10.0	5.72	8.43-11.11	9.63	4.01-7.40	5.30	0.712-0.855	0.821
5	2-18	9.5	4.4- 7.4	5.59	8.40-10.2	9.15	4.40-5.10	4.81	0.765-0.920	0.824
6	2-17	8.8	2.1- 8.4	5.17	8.55-10.32	9.18	3.78-5.04	4.46	0.725-0.835	0.790
7	4-19	10.3	4.6- 7.2	5.47	7.39-9.32	8.85	3.90-4.90	4.35	0.736-0.842	0.794
8	4-18	12.0	4.3- 7.8	5.70	9.10-9.37	9.23 <sup>c</sup>	4.62-4.73	4.66 <sup>c</sup>	0.792-0.846	0.812 <sup>c</sup>

<sup>a</sup> Average for 15 animals.

<sup>b</sup> Average for 9 animals.

<sup>c</sup> Average for 4 Sahiwal heifers.

Institute herd milk. Further progress in lactation, however, might cause more diminution in the percentage of protein.

*Carotene and vitamin A in colostrum milk.* The data showing the variations in the carotene and vitamin A content of the colostrum milk are presented in table 3. The first day's colostrum contained more than four times

TABLE 3  
Variations in the carotene and vitamin A content of colostrum milk during the first 8 days of lactation

Days postpartum	Carotene		Vitamin A	
	Range	Av.	Range	Av.
	(γ/100 ml.)		(γ/100 ml.)	
1	33.6-153.9	85.5	63.2-571.8	313.4
2	7.7-160.7	65.1	43.2-500.9	218.6
3	12.1-130.1	49.0	58.3-358.8	204.2
4	17.2-109.0	39.9 <sup>a</sup>	63.1-438.0	157.5 <sup>a</sup>
5	13.6- 70.3	31.1 <sup>b</sup>	58.6-361.4	118.4 <sup>b</sup>
6	12.1- 42.0	23.0 <sup>b</sup>	49.0-107.8	79.5 <sup>b</sup>
7	10.4- 32.3	20.1 <sup>a</sup>	51.5-140.3	77.1 <sup>a</sup>
8	10.8- 43.7	19.7 <sup>c</sup>	47.3-111.6	70.9 <sup>c</sup>

<sup>a</sup> Average for 14 animals.

<sup>b</sup> Average for 11 animals.

<sup>c</sup> Average for 12 animals.

as much carotene and vitamin A as the eighth day's sample, which might be considered equivalent in potency to a sample of milk obtained under pasture conditions. This variation was not so pronounced, however, as that reported by some English workers (5, 13, 29). As compared to the other constituents of colostrum and milk, the drop in carotene and vitamin A obviously is more marked. It might be mentioned also that the first day's colostrum did not always contain more carotene and vitamin A than the samples obtained within 4 days postpartum, although the average values showed consistent decreases. The lower carotene values were found for the animals which received very little carotene in the ration before calving. Some of these animals also secreted correspondingly lower amounts of vitamin A. The wide individual variations that are apparent in this investigation also have been observed by other workers. The average sample of colostrum obtained from the cows on the day of parturition was found to contain 85.5  $\gamma$  of carotene and 313.4  $\gamma$  of vitamin A per 100 ml. as compared to 107  $\gamma$  of carotene and 374  $\gamma$  of vitamin A for the Cornell University dairy herd on a standard dry-cow ration (28). The vitamin A-carotene ratio remained practically constant during the 8-day experimental period, indicating the flushing of these constituents from the mammary gland after their accumulation during the dry period. The concentration of both carotene and vitamin A decreased to a greater extent during the first 4 days of lactation rather than in the next 4 days. From the standpoint of vitamin A feeding, the samples for the first 4 days assume particular importance. According to Lewis and Wilson (19), the daily intake of vitamin A for a calf should be 11,000 I.U. per 100 lb. of live-weight. On this basis, the ingestion of 3 lb. of an average sample of colostrum during the first 4 days of lactation probably would be adequate for ensuring an appreciable storage of vitamin A in the liver and a satisfactory level of carotene and vitamin A in the blood of the calf. None of the calves born from the above cows showed any signs of vitamin A deficiency. The calves received colostrum *ad libitum*; consequently, the vitamin A supply was satisfactory even though the carotene and vitamin A contents of colostrum were low in a few cases.

*Carotene and vitamin A in colostrum fat.* In order to obtain more detailed information on the carotene and vitamin A contents of the colostrum fat, values for each individual cow were determined. The results are presented in tables 4 and 5. Table 4 gives the data on the carotene content of the colostrum fat. A marked drop on the second day of lactation occurred in all but two cows (nos. 13 and 15), and a further sharp drop occurred on the third day in all of the cows except no. 4. Thereafter the decline was slow and the carotene level became almost constant by the seventh day. The average first day's colostrum contained seven times as much carotene as the average eighth day's sample. No appreciable dif-



TABLE 4  
*Variations in the carotene content of colostrum fat during  
the first 8 days of lactation*

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ carotene/g. colostrum fat)							
1a	67.5	40.7	25.5	14.6	9.3	6.7	5.6	5.6
2	29.3	19.6	6.4	5.5	3.9	3.3	3.0	.....
3b	5.7	5.1	4.5	4.6	3.6	3.2	2.3	2.5
4	24.8	8.0	18.6	5.7	.....	.....	6.1	.....
5	28.5	9.8	4.9	.....	.....	.....	2.7	2.5
6	31.8	22.5	8.0	4.7	.....	.....	2.3	2.1
7	27.9	16.8	8.4	5.2	4.5	4.0	4.1	3.9
8	18.1	10.2	9.8	5.8	5.3	5.3	4.7	3.7
9b	5.4	3.6	2.7	3.5	5.4	5.7	3.3	3.4
10b	20.9	16.2	3.9	3.4	2.6	3.8	3.0	2.9
11	20.2	14.5	8.3	6.8	3.1	3.1	2.0	.....
12	25.7	15.8	10.6	10.9	7.3	8.8	4.7	2.7
13	14.7	17.1	8.7	7.2	4.1	2.4	3.3	3.9
14	23.1	14.4	8.0	8.1	9.5	5.0	5.2	5.6
15b	8.2	18.0	12.2	8.1	.....	.....	.....	2.4
Av.	23.5	15.5	9.4	6.7	5.3	4.7	3.7	3.4

\* Received comparatively large quantity of green fodders before calving because of the monsoon months.

<sup>b</sup> Received very little carotene in the ration before calving because of the drought.

ference was noted between the Hariana and the Sahiwal breeds in regard to their ability to secrete carotene in butterfat. Owing to the small number of animals, the effect of feed on the carotene content of the butterfat could not be studied thoroughly. However, an examination of the data in

TABLE 5  
*Variations in the vitamin A content of colostrum fat during the first 8 days of lactation*

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ vitamin A/g. colostrum fat)							
1	147.7	77.9	52.0	39.0	28.0	22.0	17.5	16.2
2	72.6	60.0	20.9	17.1	14.3	14.2	12.8	.....
3	41.0	45.4	52.1	15.8	15.3	15.0	11.7	11.7
4	69.4	43.1	50.0	24.5	.....	.....	22.7	.....
5	89.1	38.6	23.6	.....	.....	.....	23.0	18.0
6	106.0	75.9	28.1	11.9	.....	.....	11.1	10.1
7	82.9	47.3	29.2	21.1	15.5	11.0	11.0	11.2
8	49.8	25.2	39.7	25.4	18.3	15.1	13.2	12.8
9	30.2	17.4	18.2	17.9	14.2	12.3	13.2	12.7
10	24.3	33.2	29.0	45.0	13.4	9.8	8.9	8.6
11	76.7	60.4	48.6	27.9	24.0	19.2	13.9	.....
12	94.8	52.0	44.3	43.8	49.5	46.4	15.2	12.3
13	116.7	127.0	56.8	36.3	20.6	13.7	13.0	13.1
14	117.2	78.1	50.5	12.9	13.7	12.6	11.2	12.0
15	80.0	64.5	53.0	25.2	.....	.....	.....	11.0
Av.	79.9	56.4	39.7	26.0	20.6	17.4	14.2	12.5

table 4 reveals that there was no consistent relationship between the carotene intake and the carotene content of the colostrum fat from all the animals except for a few. Animals 3, 9, 10 and 15 secreted very small amounts of carotene, whereas animal no. 1 secreted a comparatively large amount in the colostrum fat. The first four animals were on a carotene-poor ration due to the drought period, whereas the fifth one received large quantities of green feed due to the periodic monsoon. These results tend to show that, in spite of a number of variables, the carotene content of colostrum fat also is affected, like butterfat, by the type of feed.

The data in table 5 show the decrease in the vitamin A content of the colostrum fat during the first 8 days. The average first day's colostrum contained more than six times as much vitamin A as the average eighth day's sample. These results compare favorably with those of Semb *et al.* (26), who observed that this ratio varied from five to fifteen. Although a few of the animals secreted less vitamin A in the colostrum on the first day than on the second, this was not generally the case in subsequent samples. The change in the vitamin A content as a result of the dry ration was not so apparent as it was in the case of carotene. This might be explained on the basis of the relative ease with which carotene is mobilized as compared to vitamin A. Although there was no difference between the average carotene content of the colostrum fat from first-calf heifers and cows, the vitamin A content tended to be higher in the case of the former. It is difficult to say definitely, under the present experimental conditions, whether or not first-calf heifers secrete more vitamin A in colostrum fat than do cows, an observation also made by Dann (5) and Henry *et al.* (13).

Although the numerical values reported in this investigation are not the same as those found by other investigators, there is some parallelism in the findings, especially when due consideration is given to such differences as diet, breed, and environment.

#### SUMMARY

Colostrum samples from Haryana and Sahiwal cows have been analyzed for the percentage composition of carotene, vitamin A, fat, solids-not-fat, protein and ash.

1. The colostrum contains more solids-not-fat, protein, and ash than does the normal milk.

2. Colostrum contains more than four times as much carotene and vitamin A as milk.

3. Colostrum fat was found to be six to seven times richer in carotene and vitamin A than the fat of normal milk, but both of these constituents decreased markedly during the first week postpartum. The decrease thereafter was relatively slow and carotene appeared to be affected more than vitamin A by the type of ration fed to the animals.

4. The carotene content of colostrum fat of first-calf Sahiwal heifers was comparable to that secreted by the Hariana cows, but the heifers secreted more vitamin A in the colostrum fat than did the cows.

The author is indebted to Dr. K. C. Sen for generously supplying all of the facilities needed in the course of this investigation. Further acknowledgment is made to Prof. C. W. Duncan, Department of Agricultural Chemistry, Michigan State College, for many helpful suggestions and advice in the preparation of this manuscript.

#### REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Method of Analysis. 5th Ed. Washington, D. C. 1940.
- (2) BARRON, N. S. Vitamin A and Its Relationship to Some of the Commoner Diseases of Farm Animals. *Vet. Record*, 54: 29-39. 1942.
- (3) CONVERSE, H. T., AND MEIGS, E. B. Carotene and Vitamin A in the Nutrition of Dairy Calves. *Holstein-Friesian World*, 32: 79, 98. 1935.
- (4) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. *Biochem. J.*, 26: 1072-1080. 1932.
- (5) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. II. The Carotene and Vitamin A Content of Cow's Colostrum. *Biochem. J.*, 27: 1998-2005. 1933.
- (6) DAVIS, W. L. The Chemistry of Milk. D. VanNostrand Company, Inc., New York, N. Y. 1936.
- (7) DRUMMOND, J. C., COWARD, K. H., AND WATSON, A. F. Researches on Vitamin A. VII. Notes on the Factors Influencing the Value of Milk and Butter as Sources of Vitamin A. *Biochem. J.*, 15: 540-552. 1921.
- (8) FLORA, C. C., WARD, R. E., BECHDEL, S. I., GUERRANT, N. B., AND DUTCHER, R. A. Carotene in the Nutrition of Dairy Calves. *J. Dairy Sci.*, 22: 321-328. 1939.
- (9) GILLAM, A. E., HEILBRON, I. M., FERGUSON, W. S., AND WATSON S. J. Variations in the Carotene and Vitamin A Values of the Milk Fat (Butter) of Cattle of Typical English Breeds. *Biochem. J.*, 30: 1728-1734. 1936.
- (10) GUILBERT, H. R., AND HART, G. H. Storage of Vitamin A in Cattle. *J. Nutrition*, 8: 25-44. 1934.
- (11) GULLICKSON, T. W., AND FITCH, J. B. Effect of Adding Cod Liver Oil to the Rations of Dairy Calves. *J. Dairy Sci.*, 27: 331-335. 1944.
- (12) HANSEN, R. G., PHILLIPS P. H., AND RUPEL, I. W. The Effect of Vitamin Supplements upon Survival of New-born Calves. *J. Dairy Sci.*, 29: 761-766. 1946.
- (13) HENRY, K. M., HOUSTON, J., AND KON, S. K. The Vitamin A and Carotene Content of Shorthorn Colostrum. *J. Dairy Research*, 11: 1-8. 1940.
- (14) JONES, I. R., ECKLES, C. H., AND PALMER, L. S. The Role of Vitamin A in the Nutrition of Calves. *J. Dairy Sci.*, 9: 119-139. 1926.
- (15) KEENER, H. A., BECHDEL, S. I., GUERRANT, N. B., AND THORP, W. T. S. Carotene in Calf Nutrition. *J. Dairy Sci.*, 25: 571-588. 1942.
- (16) KRAMER, M. M., BAIR, M. D., KUNERTH, B. L., AND RIDDELL, W. H. The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single Feeding Method. *J. Agr. Research*, 56: 227-232. 1938.
- (17) KRAUSS, W. E., MONROE, C. F., AND HAYDEN, C. C. The Value of a Milk Fat Substitute-skimmilk Combination for Raising Bull Calves for Veal and Heifer Calves for Replacement. *Ohio Agr. Expt. Sta. Spec. Circ.* 57. 1939.



- (18) KUHLMAN, A. H., GALLUP, W. D., AND WEAVER, E. "Carotene" in Rations for Dairy Calves. *J. Dairy Sci.*, 18: 434. 1935.
- (19) LEWIS, J. M., AND WILSON, L. T. Vitamin A Requirements in Calves. *J. Nutrition*, 30: 467-475. 1945.
- (20) LUECKE, R. W., DUNCAN, C. W., AND ELY, R. E. Milk Studies. I. Some Vitamin and Trace Elements Found in the Colostrum of the Dairy Cow, Beef Cow and Swine. *Arch. Biochem.*, 13: 277-282. 1947.
- (21) MOORE, L. A. Relationship between Carotene, Blindness due to Constriction of the Optic Nerve, Papillary Edema, and Night Blindness in Calves. *J. Dairy Sci.*, 21: 114. 1938.
- (22) MOORE, L. A., AND BERRY, M. H. Effect of Colostrum on the Vitamin A and Carotene Content of Blood Plasma of New-born Calves. *J. Dairy Sci.*, 27: 867-873. 1944.
- (23) NELSON, H. F., MOORE, L. A., HORWOOD, R. E., AND BRANAMAN, G. A. Vitamin A and Carotene Content of the Blood Plasma of Beef and Dairy Calves from Birth to Four Months of Age. *Mich. Agr. Expt. Sta. Quart. Bull.*, 27: 50-53. 1944.
- (24) PHILLIPS, P. H., LUNDQUIST, N. S., AND BOYER, P. D. The Effect of Vitamin A and Certain Members of the B-Complex upon Calf Scours. *J. Dairy Sci.*, 24: 977-982. 1941.
- (25) RAY SARKAR, B. C. Effect of Season, Breed and Species of Ruminants on the Vitamin A Potency of Butterfat. *J. Dairy Sci.*, 31: 165-172. 1948.
- (26) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat Soluble Vitamins. XLI. The Carotene and Vitamin A Content of Colostrum. *J. Biol. Chem.*, 107: 697-703. 1934.
- (27) SOLDATENKOV, P. F. The Influence of Vitamin A on the Development of Calves. *Problemy Zhivotnovod.*, 8: 80-83. 1935. (Cited in *Chem. Abstracts*, 30: 2613. 1936.)
- (28) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., WHITING, F., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (29) STEWART, J., AND MCCALLUM, J. W. The Vitamin A Content of the Colostrum of Dairy Cows. *J. Agr. Sci.*, 28: 428-436. 1938.
- (30) STEWART, J., AND MCCALLUM, J. W. "White Scour" in Calves and Related Infections. I. The Significance of the Vitamin A Content of the Colostrum as a Predisposing Factor in the Causation of such Conditions. *J. Comp. Path. Therap.*, 51: 290-295. 1938.
- (31) STEWART, J., AND MCCALLUM, J. W. The Effect of a Vitamin A-rich Diet on the Vitamin A Content of the Colostrum of Dairy Cows. *J. Dairy Research*, 13: 1-4. 1942.



# COMPARATIVE ANTIRACHITIC VALUE OF FIELD-CURED HAY, BARN-DRIED HAY, AND WILTED GRASS SILAGE FOR GROWING DAIRY CALVES

L. A. MOORE, J. W. THOMAS, W. C. JACOBSON, C. G. MELIN,  
AND J. B. SHEPHERD

*Bureau of Dairy Industry, Agricultural Research Administration,  
United States Department of Agriculture*

According to present opinion, hay crops contain little or no vitamin D before they are cut. It is only after the crop is cut and while it is exposed to the rays of the sun during the curing process that activation of certain plant sterols takes place to form vitamin D.

With the newer methods of conserving hay crops, such as curing the hay in the barn or making wilted silage, the time of exposure to the rays of the sun is less than when the crop is cured in the field. Consequently, when barn-cured hay or wilted silage is the sole source of vitamin D for calves, it might be questionable whether they would obtain enough of the vitamin to meet their requirements. A review of the literature does not supply a direct answer to this question.

Some information on the vitamin D content of forage plants subjected to various curing processes, as determined by rat bioassays, is available. Steenbock *et al.* (9) reported that clover leaves cured without exposure to direct sunlight showed no vitamin D activity when fed to rats at the 1- and 5-per cent levels. On the other hand, leaves from the same field that were cured in the sun and fed on the same basis showed definite vitamin D activity. In later experiments Hart *et al.* (4) found that alfalfa hay cured in Colorado with limited exposure to the sun contained some vitamin D but less than hay cured with full exposure to the sun. Russell (7) reported some vitamin D activity in alfalfa leaves cured out of sunlight but considerably less than in leaves cured in the sun or leaves cured in the sun and irradiated. Smith and Briggs (8) reported very little vitamin D activity of alfalfa leaves cured in the dark. Leaves cured for 15 hours in sunlight had considerable activity but not so much as leaves exposed for 57 hours. However, Hodgson and Knott (5) found that an artificially dehydrated pasture mixture of English ryegrass, Italian ryegrass, and white clover from irrigated land had as much calcifying activity as the same material sun cured. Wallis (10) reported a considerable increase in the vitamin D activity of alfalfa hay after sun curing. However, considerable variation was found between crops in this respect, and it was concluded that "there are other influences than the amount of sunshine received which greatly affect the vitamin D content of the resulting hay." One

Received for publication January 28, 1948.



sample of hay cut after dark and dried artificially contained 812 I.U. per pound. This value is equal to or greater than some of the values reported for sun-cured hay. Bechdel *et al.* (1) found that artificially dried alfalfa contained considerably less vitamin D than sun-cured hay. These same workers report values of 150 to 300 USP units of vitamin D per pound in two different lots of night-harvested dehydrated alfalfa hay. If such a hay were fed to calves to the extent of their roughage requirements, it should furnish the minimum requirements.

Some experimental data on the antirachitic value of hay for calves have been reported in the literature. Huffman *et al.* (6) found that 2 lb. per day of sun-cured timothy prevented rickets up to 1 year of age and 3 lb. per day cured rickets in a 9-month-old calf. Two pounds of sun-cured alfalfa hay per day prevented rickets in one calf up to 195 days of age. In using the curative method, these workers were unable to obtain a sufficient intake of timothy hay cured in the dark to determine its antirachitic effect. In studying the data of these workers, one comes to the conclusion that about 0.7 lb. of sun-cured hay per 100 lb. of body weight is about a minimum for the prevention of rickets in growing calves.

The Pennsylvania Agricultural Experiment Station (2) found that a mild rachitic condition developed in a 6-month feeding period with 1 lb. of sun-cured alfalfa hay per day added to the basal diet, whereas on the same basis artificially cured alfalfa permitted a severe rachitic condition to develop. Two and one-half pounds of good sun-cured alfalfa hay per day prevented the development of a rachitic condition during a 6-month period, whereas the same quantity of artificially cured hay permitted the development of a mild rachitic condition.

The review of literature gives little information for making practical recommendations on the question of whether vitamin D supplements should be used when barn-cured hay or wilted silage is fed as the sole source of vitamin D for calves. Results reported in the literature on rat bioassays with hays show that hay cured without exposure to the sun contains considerably less vitamin D than sun-cured hay. The data with calves likewise give the same indication. In the experiments thus far conducted with calves, limited quantities of hay were fed to bring out differences between sun curing and artificial curing. This raises the question of whether the artificially dried hay would not have furnished sufficient vitamin D for calves had it been fed according to body weight or the appetite of the calf (2 to 2.5 lb. per 100 lb). For this reason the data on artificially cured hay cannot be used in making practical recommendations for vitamin D supplementation either for artificially cured hay, barn-cured hay, or wilted silage.

The present study was undertaken to determine whether barn-cured hay or wilted silage will supply sufficient vitamin D to growing calves when it

is fed at adequate levels, *i.e.*, at levels which ordinarily would be fed under practical feeding conditions.

#### EXPERIMENTAL PROCEDURE

In the summer of 1945 a second cutting of alfalfa was harvested simultaneously as field-cured hay, barn-cured hay, and wilted silage. Good weather conditions prevailed during the time the crop was being harvested by the three methods, so there was a maximum exposure to the sun during daylight hours. The wilted silage was exposed for 4 to 6 hours between sunrise and sunset, the barn-cured hay for 12 to 16 hours, and the field-cured hay for 30 to 40 hours, although there was considerable variation in this respect.

Holstein and Jersey male calves were reared to 90 days of age on a ration of skim milk, grain, alfalfa hay and cod-liver oil. In addition, it was necessary to use two crossbred calves, one of which was placed in the Holstein group and one in the Jersey group. Flaxseed jelly, corn meal, or grain were added to the skim milk, beginning when the calves were about 10 days of age, in order to increase the energy intake. Skim milk was discontinued at 30 days of age for the Holsteins and at 45 days for the Jersey calves. Three calves on the experiment (503, 701, 703) received whole milk to 60 days of age, along with alfalfa hay and grain.

At 90 days of age the calves were placed on the basal ration made up as follows: Corn meal, 60 parts; wheat bran, 30 parts; soybean meal, 20 parts; linseed meal, 10 parts; iodized salt, 1 part; calcium carbonate, 2 parts. In addition, 1 lb. of beet pulp per 100 lb. of body weight, 100 g. of dehydrated alfalfa leaf meal, and 4 lb. of skim milk were fed daily. The calves were kept on this ration for a period of 50 days or until they were 140 days of age in order to deplete their vitamin D stores. Calcium, phosphorus and phosphatase values of the blood were used to measure depletion. Following the depletion period the calves were fed, in addition to the basal grain ration, the particular experimental forage they were to receive for a period of 180 days. In some instances it was necessary to place the calves on their respective forages before the end of the 50-day depletion period because of blood values which indicated the incipient stage of rickets. The calves were kept in a darkened barn out of direct sunlight. They were turned to a dry lot for exercise at night.

Groups of six calves each were fed the alfalfa forage cured by the three different methods. Within each group three different levels of forage were fed with two calves on each level (table 1). The Jersey and Holstein calves were distributed equally between and within groups.

The wilted silage was fed on a hay-equivalent basis, taking into consideration the moisture content. The calves received, in addition to the basal grain ration and the specified forage, 4 lb. of skim milk daily. Total

digestible nutrients were fed according to the Morrison standard by adjusting the grain intake after allowing for the T.D.N. in the skim milk and forage. Adjustments of forage and grain were made each 2 weeks.

Two positive control calves were continued on the depletion ration but received 10,200 USP units of vitamin D daily in the form of irradiated yeast after the 50-day depletion period. One negative control animal was used which received the depletion ration but no vitamin D.

After the calves received the forage for 180 days they were slaughtered and the eighth and ninth ribs were saved for ash analysis. Ash determinations were made on the distal 10 per cent of the two ribs after they were subjected to hot alcohol extraction. The calcium, inorganic phosphorus and phosphatase contents of the blood were determined each week, except toward the end of the experiment, when the determinations were made each 2 weeks.

Rat bioassays for vitamin D were made on the forage put up by the

TABLE 1  
*Rate of forage feeding per 100 lb. of body weight*

No. of calves	Field-cured hay (Group 1)	Barn-cured hay (Group 2)	Wilted silage (hay equivalent) (Group 3)
	(lb.)	(lb.)	(lb.)
2	0.5	0.7	0.7
2	1.0	1.2	1.2
2	1.5	1.7	1.7

three procedures in order to obtain comparative values. The usual line test procedure was used by including 10 per cent of the forage in the basal rachitogenic diet.

In 1946 another crop of wilted alfalfa silage was fed to two calves, beginning as soon after birth as the calves would consume the silage. They received a limited quantity of whole milk to 60 days of age, but after this time their sole source of vitamin D was from the wilted alfalfa silage. The silage was fed on a hay-equivalent basis of 1.5 lb. per 100 lb. of body weight. The two calves were slaughtered at 8 and 9 months of age.

#### RESULTS AND DISCUSSION

The effect of feeding alfalfa cured by the three different methods on rate of growth is shown in table 2. These data show that the best rate of gain was made by the calves on the wilted silage, their average daily gain being 1.71 lb. per day for the 180-day period. The next best gain was by the calves on barn-cured hay, which averaged 1.65 lb. a day, whereas the field-cured hay produced a daily gain of 1.48 lb. Since the feed intake was well-controlled, these results indicate that good gains can be obtained with



TABLE 2

*Gain in weight on field-cured and barn-cured hay and on wilted silage  
(180-day feeding period)*

Field-cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate <sup>a</sup>	Total gain	Calf no.	Rate <sup>a</sup>	Total gain	Calf no.	Rate <sup>a</sup>	Total gain
	(lb.)	(lb.)		(lb.)	(lb.)		(lb.)	(lb.)
705-H <sup>b</sup>	0.5	330	503-H	0.7	270	703-H	0.7	350
2380-J	0.5	218	2384-J	0.7	288	2379-J	0.7	259
250-H	1.0	300	2557-H	1.2	344	120-H	1.2	330
504-J	1.0	279	2385-J	1.2	266	505-J	1.2	263
330-X	1.5	279	2558-H	1.7	301	701-H	1.7	342
2383-J	1.5	248	332-X	1.7	314	506-J	1.7	282
Total av. gain		267	.....	.....	297	.....	.....	307
Av. daily gain		1.48	.....	.....	1.65	.....	.....	1.71

<sup>a</sup> Rate=hay or hay equivalent daily per 100 lb. of body weight.

<sup>b</sup> H=Holstein; J=Jersey; X=Crossbred.

wilted silage. There was very little feed refusal by the two calves that were fed at the highest level (an equivalent of 1.7 lb. of hay per day per 100 lb. of body weight). One Holstein calf, weighing 650 lb., consumed as much as 30 lb. of wilted silage per day, which was the sole roughage. The calves on the wilted silage were very sleek in appearance and appeared to do well throughout the experiment.

The results of the ash analyses of the distal 10 per cent of the eighth and ninth ribs are shown in table 3. The results of the analyses of the two ribs were averaged. These results show that all three forages possessed definite antirachitic properties for calves. There does not appear to be any dif-

TABLE 3

*The ash values of rib ends*

Field-cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate <sup>a</sup>	Ash	Calf no.	Rate <sup>a</sup>	Ash	Calf no.	Rate <sup>a</sup>	Ash
	(lb.)	(%)		(lb.)	(%)		(lb.)	(%)
705-H	0.5	53.1 N <sup>b</sup>	503-H	0.7	50.9 Sl.st	703-H	0.7	53.0 N
2380-J	0.5	55.8 Sl.st	2384-J	0.7	56.6 N	2379-J	0.7	55.0 St
250-H	1.0	56.5 Sl.st	2557-H	1.2	61.1 Sl.st	120-H	1.2	54.9 St
504-J	1.0	56.7 N	2385-J	1.2	56.2 Sl.st	505-J	1.2	59.0 N
330-X	1.5	56.0 N	2558-H	1.7	59.4 Sl.st	701-H	1.7	57.1 Sl.st
2383-J	1.5	61.2 N	332-X	1.7	58.8 N	506-J	1.7	62.0 N
Negative								
2570-H		39.0 St						
Positive			2571-H		50.8 Sl.st			
Positive			508-J		51.6 N			

<sup>a</sup> Rate=hay or hay equivalent per day per 100 lb. of body weight.

<sup>b</sup> N=normal; Sl.st=slightly stiff; St=stiff.

ference in this respect between the three lots of calves that were fed the three different kinds of forage. In all three groups, the calves that were fed at the lowest roughage level showed the lowest ash values. The ash value for the negative control was only 39 per cent and it was necessary to remove this calf from the experiment after 160 days because of the extreme rachitic condition. The ash values for the two positive control animals were not so high as for the calves that were fed the various levels of forage, even

TABLE 4

*The effect of feeding 1.5 lb. field-cured hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase  
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
95	8.1	8.1	14.3
102	7.0	6.2	20.6
109	6.7	6.4	18.2
116	7.1	4.8	17.2
<i>Hay added</i>			
122	8.0	5.8	18.2
130	7.8	4.5	17.9
136	8.1	5.8	9.4
147	9.0	6.8	18.8
161	11.3	8.4	12.4
175	11.3	9.3	10.7
189	11.4	9.3	9.9
196	10.3	8.0	9.2
218	10.6	6.4	10.4
231	9.3	6.9	9.9
245	10.5	7.6	9.2
259	10.0	5.8	5.8
274	10.1	7.4	6.6
287	10.4	8.6	5.0
302	10.0	8.7	5.7
316	9.6	8.1	3.9

though the controls were fed 10,200 USP units of vitamin D per day. The calcium intake of these two calves was not so high as for the calves receiving forage, since no extra calcium was fed, yet the intake at the end of the experimental period was as much as 18 g., or well above a 10-g.-minimum. Therefore, forages may contain factors other than vitamin D which aid in calcification.

The calves were examined periodically for evidence of stiffness (table 3). While there was less stiffness or indication of clinical rickets in the group that received field-cured hay, between groups the differences probably are not significant. There did not appear to be a direct correlation between stiffness and the bone ash values. It also was noted that the calves that

showed stiffness did not show abnormal blood values for calcium, inorganic phosphorus and phosphatase at the end of the experiment. During the depletion period, however, abnormal blood values for calcium, phosphorus and phosphatase usually preceded the clinical signs of rickets.

All the detailed data of blood analyses for each calf cannot be presented. However, the data for one crossbred and two Holstein calves that were fed the largest intake on each kind of forage are shown in tables 4, 5

TABLE 5

*The effect of feeding 1.7 lb. barn-dried hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase  
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
91	10.9	8.8	8.4
108	11.3	8.0	10.2
115	10.5	6.8	13.9
122	8.5	7.4	14.6
128	7.6	7.7	.....
136	6.4	8.6	19.4
<i>Hay added</i>			
142	6.3	8.2	16.2
153	8.4	7.4	14.2
167	11.6	8.8	8.4
181	10.8	9.6	7.2
195	11.2	8.9	7.1
210	10.3	8.3	7.3
224	9.6	7.0	6.6
237	9.3	7.8	6.6
251	10.3	6.8	8.0
265	9.8	6.8	9.2
280	9.7	6.4	5.6
293	9.8	8.6	5.0
308	9.5	6.2	7.0
322	9.8	7.0	7.0

and 6. A study of the detailed data does not reveal any marked differences between the three groups of calves. The addition of forage in the three different forms caused the blood values to return to normal following the depletion period.

The quantity of solar radiation received by the forage, as shown in table 7, was calculated from hourly figures covering the period the forage was in the swath and windrow. The values are in terms of gram-calories per square centimeter of horizontal surface. The vitamin D content of the forage as determined by rat bioassays also is shown in the table in terms of International Units of vitamin D per g. of air-dried forage. There does not appear to be any close correlation between the amount of solar radia-



ference in this respect between the three lots of calves that were fed the three different kinds of forage. In all three groups, the calves that were fed at the lowest roughage level showed the lowest ash values. The ash value for the negative control was only 39 per cent and it was necessary to remove this calf from the experiment after 160 days because of the extreme rachitic condition. The ash values for the two positive control animals were not so high as for the calves that were fed the various levels of forage, even

TABLE 4

*The effect of feeding 1.5 lb. field-cured hay per 100 lb. body weight on blood calcium, phosphorous and phosphatase  
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
95	8.1	8.1	14.3
102	7.0	6.2	20.6
109	6.7	6.4	18.2
116	7.1	4.8	17.2
<i>Hay added</i>			
122	8.0	5.8	18.2
130	7.8	4.5	17.9
136	8.1	5.8	9.4
147	9.0	6.8	18.8
161	11.3	8.4	12.4
175	11.3	9.3	10.7
189	11.4	9.3	9.9
196	10.3	8.0	9.2
218	10.6	6.4	10.4
231	9.3	6.9	9.9
245	10.5	7.6	9.2
259	10.0	5.8	5.8
274	10.1	7.4	6.6
287	10.4	8.6	5.0
302	10.0	8.7	5.7
316	9.6	8.1	3.9

though the controls were fed 10,200 USP units of vitamin D per day. The calcium intake of these two calves was not so high as for the calves receiving forage, since no extra calcium was fed, yet the intake at the end of the experimental period was as much as 18 g., or well above a 10-g.-minimum. Therefore, forages may contain factors other than vitamin D which aid in calcification.

The calves were examined periodically for evidence of stiffness (table 3). While there was less stiffness or indication of clinical rickets in the group that received field-cured hay, between groups the differences probably are not significant. There did not appear to be a direct correlation between stiffness and the bone ash values. It also was noted that the calves that

showed stiffness did not show abnormal blood values for calcium, inorganic phosphorus and phosphatase at the end of the experiment. During the depletion period, however, abnormal blood values for calcium, phosphorus and phosphatase usually preceded the clinical signs of rickets.

All the detailed data of blood analyses for each calf cannot be presented. However, the data for one crossbred and two Holstein calves that were fed the largest intake on each kind of forage are shown in tables 4, 5

TABLE 5

*The effect of feeding 1.7 lb. barn-dried hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase  
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
91	10.9	8.8	8.4
108	11.3	8.0	10.2
115	10.5	6.8	13.9
122	8.5	7.4	14.6
128	7.6	7.7	.....
136	6.4	8.6	19.4
<i>Hay added</i>			
142	6.3	8.2	16.2
153	8.4	7.4	14.2
167	11.6	8.8	8.4
181	10.8	9.6	7.2
195	11.2	8.9	7.1
210	10.3	8.3	7.3
224	9.6	7.0	6.6
237	9.3	7.8	6.6
251	10.3	6.8	8.0
265	9.8	6.8	9.2
280	9.7	6.4	5.6
293	9.8	8.6	5.0
308	9.5	6.2	7.0
322	9.8	7.0	7.0

and 6. A study of the detailed data does not reveal any marked differences between the three groups of calves. The addition of forage in the three different forms caused the blood values to return to normal following the depletion period.

The quantity of solar radiation received by the forage, as shown in table 7, was calculated from hourly figures covering the period the forage was in the swath and windrow. The values are in terms of gram-calories per square centimeter of horizontal surface. The vitamin D content of the forage as determined by rat bioassays also is shown in the table in terms of International Units of vitamin D per g. of air-dried forage. There does not appear to be any close correlation between the amount of solar radia-

tion and the vitamin D content of the forage, although the vitamin D content in the field-cured hay was somewhat higher.

Using the figure 0.47 I.U. per g. for the barn-dried hay, when the calves were fed at the rate of 0.7 lb. of barn-dried hay per 100 lb. of body weight the intake of vitamin D would be 150 I.U. per 100 lb. of body weight. On the same basis, when the calves were fed at the rate of 1.2 lb. of barn-dried hay per 100 lb. of body weight, the vitamin D intake would be 256 I.U., and when they were fed at the rate of 1.7 lb. the intake would be 363 I.U. per

TABLE 6

*The effect of feeding 1.7 lb. hay equivalent of silage per 100 lb. body weight on blood calcium, phosphorus and phosphatase  
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
101	11.8	9.4	.....
107	10.5	9.5	.....
115	11.6	9.1	12.5
122	10.5	8.1	16.4
129	10.6	.....	17.9
136	9.4	8.2	15.0
<i>Silage added</i>			
142	10.8	7.5	16.5
150	10.6	6.8	10.6
156	11.9	7.3	8.7
167	12.1	7.9	6.9
181	.....	8.1	6.3
195	10.8	8.7	8.1
209	12.3	7.4	8.6
225	10.0	7.8	7.2
238	10.9	6.6	6.2
251	9.7	6.3	6.9
265	10.5	6.7	6.1
279	9.7	6.8	7.6
294	9.7	6.9	5.9
307	10.0	7.1	3.7
322	9.7	7.0	7.8

100 lb. of body weight. If 300 I.U. per 100 lb. of body weight is taken as the minimum requirement, it would be met by the feeding of 1.5 to 1.7 lb. of barn-dried hay per 100 lb. of body weight. On this basis, one would not expect marked rickets to develop in the calves fed wilted silage and barn-cured hay in this experiment even though the vitamin D intake was near the minimum allowance or slightly below. The observations on stiffness and bone ash values confirm this opinion.

The two calves that were fed from birth the wilted silage that was made in 1946 showed no evidence of rickets at any time during the experiment.



The calcium, phosphorus and phosphatase values of the blood remained within the normal range. When these two calves were slaughtered at 8 and 9 months of age, respectively, they were in excellent condition and had sleek hair coats. These two calves did not consume as much dry matter from wilted silage up to 90 days of age as they would be expected to consume from hay. However, after 90 days of age they easily consumed the 1.5 lb. (hay equivalent) of wilted silage offered per 100 lb. of body weight.

The vitamin D content of the three forages put up simultaneously in 1946 as determined by rat bioassays is shown in table 7. The figure 0.87 for the wilted silage was checked and found to be correct. Thus, one would not expect the two calves that were fed the wilted silage containing 0.87 I.U. per g. to develop rickets. The high vitamin D content of this lot

TABLE 7  
*Exposure of forage to solar radiation and its vitamin D content*

	Radiation exposure <sup>a</sup>			Vitamin D content
	In swath	In windrow	Total	
	(g.-cal./cm. <sup>2</sup> )	(g.-cal./cm. <sup>2</sup> )	(g.-cal./cm. <sup>2</sup> )	(I.U./g.)
<i>1945 crop</i>				
Wilted silage .....	104	117	221	0.56
Barn-dried hay .....	364	158	522	0.47
Field-cured hay .....	573	777	1350	0.97
<i>1946 crop</i>				
Wilted silage .....	134	109	243	0.87
Barn-dried hay .....	323	218	541	0.58
Field-cured hay .....	515	958	1473	0.88

<sup>a</sup> Calculated from data furnished by Dr. W. F. Shenton of American University of Washington, D. C.

of wilted silage probably was due to the presence of a larger quantity of foreign material in the crop than was present in the field-cured hay and barn-cured hay. It was necessary in 1946 to use one field which was somewhat weedy in order to have sufficient silage for the planned experiments.

Therefore, the results indicate that barn-cured hay and wilted silage conserved under the conditions of this experiment will contain sufficient vitamin D to prevent rickets in dairy calves when these forages are consumed at adequate levels. The rat bioassay data show that these forages contained less vitamin D than the field-cured hay. The difference, however, was not sufficiently great to precipitate rickets when the forages were fed at the rate of 1.5 to 1.7 lb. of hay equivalent per 100 lb. of body weight.

A greater difference in the vitamin D content of these forages might have been expected in view of the accepted concept of the mechanism of formation of vitamin D in forage crops. However, while the field curing of hay promotes an increase in its vitamin D content, there may be con-

siderable vitamin D present in the crop as it stands in the field. The amount of vitamin D probably is governed by the quantity or area of dead plant tissue, such as dead stems or leaves or partially injured leaves. The amount of dead tissue might vary, depending on such factors as climatic conditions, stage of maturity, disease, and insect injury. Thus, in the case of leaf hopper injury, minute injured areas exist on the surface of the leaf, where activation of the sterols might take place. Probably an absolutely green plant without injury of any sort would contain no vitamin D. However, under practical conditions in the eastern section of the country it is doubtful whether such a condition ever exists.

The suggestion that the vitamin D content of hay crops might be affected by the quantity of dead material in the crop at time of cutting is found in the paper of Bechtel *et al.* (3). For instance, these investigators found that, in the corn plant at the silage-making stage, the silks, tassels, and dried leaves were excellent sources of vitamin D, whereas the green part of the plant was devoid of vitamin D. The effect of some of these factors on the vitamin D content of tissues of forage plants, now is under investigation.

While it seems probable that wilted silage and barn-cured hay contain sufficient vitamin D so that no supplementary feeding of vitamin D is needed for calves kept out of direct sunlight, further fundamental studies on the factors affecting the vitamin D content of plant tissue as it stands in the field at the hay stage need to be carried out. It would seem quite probable that a higher vitamin D intake might be possible where a good quality of barn-cured hay or wilted silage is fed than where a poor quality of field-cured hay is fed, because of the greater palatability of the former.

Rickets has been reported in dairy calves under practical farm conditions where grain was fed in excess so that very little sun-cured hay was consumed. Vitamin A and calcium deficiency also probably would be present where such a feeding practice is being used. It would seem more logical to advocate proper management practices to correct such conditions rather than the addition of supplements to the grain mixtures fed. Limiting the grain fed so that hay consumption could be increased and exposure of the calves to sun would be of benefit. Calves exposed to direct sunlight during the winter months and receiving no other source of vitamin D do not develop rickets at Beltsville.

Vitamin D sometimes is added to commercial grain mixtures for mature dairy cattle. While the cost is low, it is the opinion of the authors that there are not sufficient concrete scientific data at the present time to warrant such a practice. Later developments may justify such supplementation, but scientific fiction is not sufficient justification for such a practice.

#### SUMMARY AND CONCLUSIONS

1. Wilted silage fed as the sole roughage to growing dairy calves produced gains as good or better than those observed in calves fed barn-cured

and field-cured hay. The calves that were fed the wilted silage were sleek and excellent in appearance.

2. Wilted silage, made in two different years, contained sufficient vitamin D to prevent rickets in growing calves that were kept out of sunlight when the silage was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight on the hay-equivalent basis (3 to 4 lb. per 100 lb. body weight on the silage basis).

3. Barn-cured hay made one year contained sufficient vitamin D to prevent rickets in calves that were kept out of sunlight when it was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight.

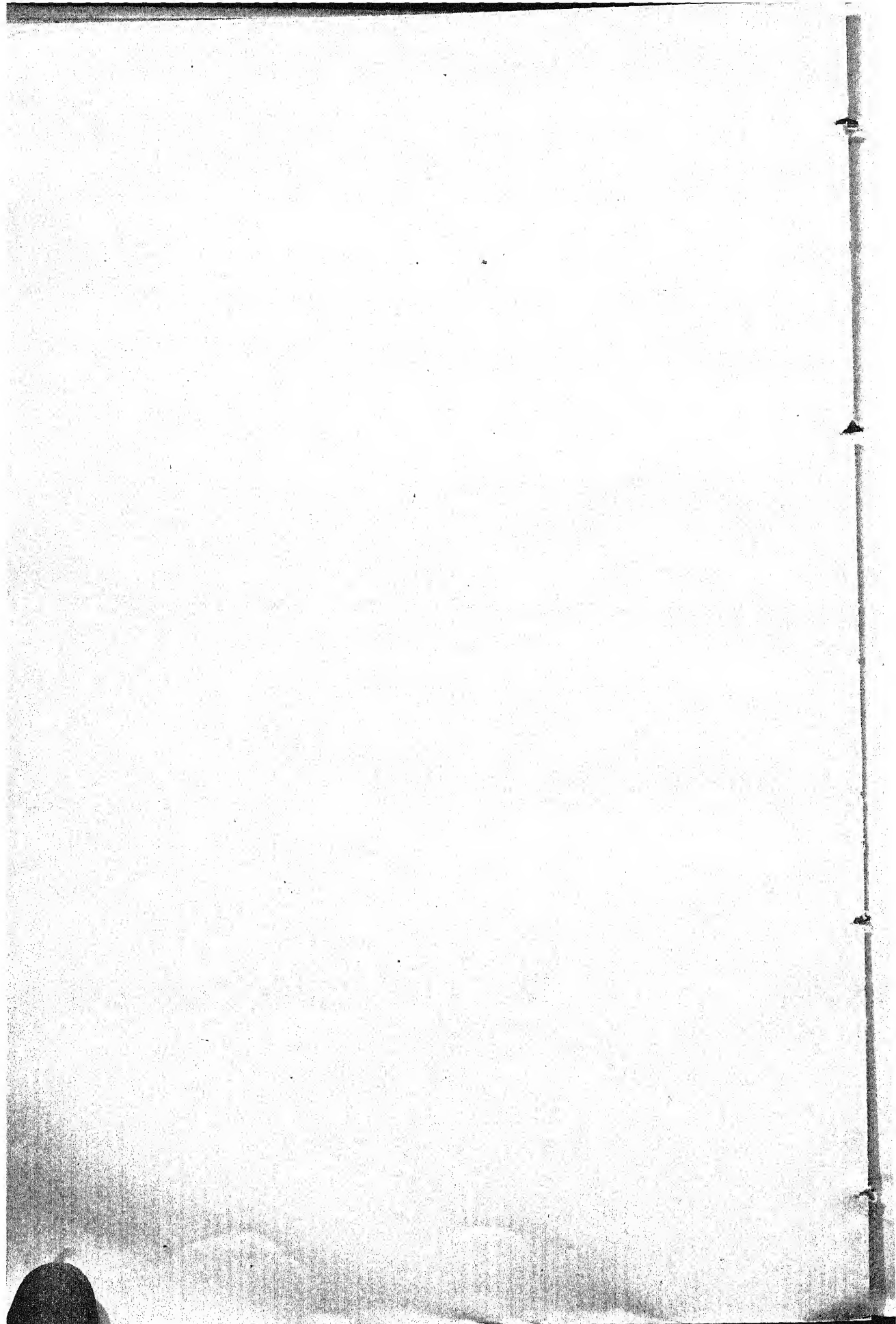
4. Rat bioassays of the forages fed for vitamin D, which confirmed the results of the calf-feeding experiment, showed that they contained sufficient vitamin D to prevent rickets in growing calves.

5. While further fundamental data must be collected on the factors affecting the vitamin D content of forages harvested with a minimum exposure to the sun, it seems quite likely that barn-cured hay and wilted silage, at least as conserved under Beltsville conditions, contain sufficient vitamin D for growing calves to prevent rickets if fed at the usual levels of roughage feeding.

#### REFERENCES

- (1) BECHDEL, S. I., HILSTON, N. W., GUERRANT, N. B., AND DUTCHER, R. A. The Vitamin D Requirement of Dairy Calves. Pa. Agr. Expt. Sta. Bull. 364. 1938.
- (2) BECHDEL, S. I., LANDSBERG, K. G., AND HILL, O. J. Rickets in Calves. Pa. Agr. Expt. Sta. Bull. 291. 1933.
- (3) BECHDEL, H. E., HUFFMAN, C. F., DUNCAN, C. W., AND HOPPERT, C. A. Vitamin D Studies in Cattle. IV. Corn Silage as a Source of Vitamin D for Dairy Cattle. J. Dairy Sci., 19: 359-372. 1936.
- (4) HART, E. B., STEENBOCK, H., TEUT, E. C., AND HUMPHREY, G. C. Dietary Factors Influencing Calcium Assimilation. XII. A Study of the Influence of Hays Cured with Varying Exposure to Sunlight on the Calcium Metabolism of Milking Cows. J. Biol. Chem., 84: 367-376. 1929.
- (5) HODGSON, R. E., AND KNOTT, J. C. The Calcifying Properties of Green, Artificially Dried, and Sun-cured Pasture Herbage. J. Agr. Research, 48: 439-446. 1934.
- (6) HUFFMAN, C. F., DUNCAN, C. W., AND LIGHTFOOT, C. C. Vitamin D Studies in Cattle. I. The Antirachitic Value of Hay in the Ration of Dairy Cattle. J. Dairy Sci., 18: 511-526. 1935.
- (7) RUSSELL, W. C. The Effect of the Curing Process upon the Vitamin A and D Content of Alfalfa. J. Biol. Chem., 85: 289-297. 1929.
- (8) SMITH, M. C., AND BRIGGS, I. A. The Antirachitic Value of Alfalfa as Affected by Exposure to Sunshine in the Curing Process. J. Agr. Research, 46: 235-240. 1933.
- (9) STEENBOCK, H., HART, E. B., ELVEHJEM, C. A., AND KLETZIEN, S. W. F. Dietary Factors Influencing Calcium Assimilation. VI. The Antirachitic Properties of Hays as Related to Climatic Conditions with some Observations on the Effect of Irradiation with Ultraviolet Light. J. Biol. Chem., 66: 425-440. 1925.
- (10) WALLIS, G. C. Vitamin D Deficiency in Dairy Cows. S. Dak. Agr. Expt. Sta. Bull. 372. 1944.





## THE EFFECT OF STREPTOMYCIN UPON THE LIVABILITY AND BACTERIAL CONTENT OF BOVINE SEMEN<sup>1</sup>

J. O. ALMQUIST,<sup>2</sup> P. J. GLANTZ,<sup>3</sup> AND W. T. S. THORP<sup>4</sup>

*Pennsylvania Agricultural Experiment Station, State College*

Various antibacterial agents have been used in efforts to overcome the problems associated with the presence of bacteria in bull semen used for artificial breeding (1, 4, 5, 6). Earlier investigations at this Experiment Station (1) upon the use of penicillin in diluted bull semen showed that certain organisms were resistant to penicillin at levels as high as 2,000 units per ml. Since streptomycin inhibits the growth of a number of organisms which are insusceptible or only slightly susceptible to penicillin, it seemed desirable to study its effect on bacteria commonly found in bull semen.

Gunsalus *et al.* (2, 3) have reported that bulls harboring *Pseudomonas aeruginosa* in their reproductive tracts were apt to have low breeding efficiencies and be poor risks for use in artificial breeding. Since Waksman and Reilly (7) have found streptomycin to be bactericidal for *Pseudomonas aeruginosa*, its addition to semen might restore normal breeding efficiency to bulls of lowered fertility known to disseminate this organism in their semen.

### EXPERIMENTAL

*Effect of streptomycin upon the livability of spermatozoa.* In a preliminary study to determine the relative resistance of stored bull spermatozoa to streptomycin, this antibiotic was added to four ejaculates diluted 1:24 with yolk-citrate diluter at levels of 100, 500, 1,000, 1,500, 2,000, 2,500, 5,000 and 10,000 units or  $\gamma$  per ml. of diluted semen. When compared to untreated controls, no marked differences in spermatozoan livability were noted during the 20-day storage period in the levels ranging from 100 to 1,500  $\gamma$ . However, concentrations of 2,500, 5,000 and 10,000  $\gamma$  per ml. of diluted semen greatly reduced motility during storage. On the basis of these results, streptomycin was added to ten samples of bull semen at the rate of 100, 250, 500, 750, 1,000, 1,250, 1,500 and 2,000  $\gamma$  per ml. of diluted semen with appropriate controls. Each of the ten ejaculates was diluted 1:24 with yolk-citrate diluter composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g.

Received for publication February 4, 1948.

<sup>1</sup> Authorized for publication February 2, 1948, as paper no. 1428 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This study was supported in part by a research grant from Charles Pfizer and Co., Inc., Brooklyn, New York, who also provided the streptomycin.

<sup>2</sup> Department of Dairy Husbandry.

<sup>3</sup> Animal Pathology Laboratory, Department of Animal Husbandry.

<sup>4</sup> Now with the National Institute of Health, Bethesda, Maryland.

of sodium citrate dihydrate in 100 ml. of water distilled over glass. The streptomycin powder was dissolved in sterile sodium citrate solution and mixed with egg yolk to provide a diluter with a 1:1 ratio of yolk to buffer.

The diluted samples were stored at 4.5° C. and the percentages of motile spermatozoa were determined every 2 days for 20 days. In order to avoid bias on the part of the observer making the motility estimations, randomized numbers were placed on the test tubes of diluted semen containing the various levels of streptomycin. Bacterial counts and streptomycin assays were made on these samples after 0, 8 and 16 days of storage.

The ten ejaculates had a mean concentration of 1,054,000 spermatozoa per cubic millimeter, a mean initial motility of 69 per cent active spermatozoa, and a mean methylene blue reduction time of 9.4 minutes.

The mean motility data for the ten ejaculates are shown in table 1.

TABLE 1  
*The effect of streptomycin upon the livability of bovine spermatozoa*  
(Mean of 10 determinations)

Streptomycin units per ml. of diluted semen	Per cent motile spermatozoa					
	Before storage	After storage at 4.5° C. for				
		4 days	8 days	12 days	16 days	20 days
Control	69	59	44	33	16	10
100	69	57	47	33	19	8
250	69	58	47	34	17	8
500	69	61	47	34	19	5
750	69	59	46	31	18	5
1000	69	59	45	32	15	9
1250	69	59	45	25	14	3
1500	69	60	42	27	13	6
2000	69	60	43	25	14	4

Using the observations made at each 2-day interval, analysis of variance showed no significant differences in spermatozoan livability between levels of streptomycin of 0, 100, 250, 500, 750 and 1,000  $\gamma$  per ml. However, the three highest levels (1,250, 1,500 and 2,000  $\gamma$  per ml. of diluted semen) brought about a highly significant decrease in livability as compared to untreated diluted semen.

The relationship between spermatozoan livability and concentration of streptomycin was studied further by means of regression. While both highly significant linear and curvilinear regressions were calculated, a test for significance of departure from linearity showed that a straight line was more applicable to the livability data (fig. 1.). Compared to untreated control samples, the mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250  $\gamma$  of streptomycin.

*Effect of streptomycin upon the bacterial content of diluted semen.* Bacterial plate counts were determined on nine samples of diluted semen



after 0, 8 and 16 days of storage using veal infusion agar containing 4 per cent sterile defibrinated ox blood. The samples were plated in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 and incubated for 48 hours at 37° C. Desoxycholate agar plates incubated at 37° C. were used for determining the number of bacteria belonging to the coliform group. The same procedure was followed in obtaining bacterial counts on portions of undiluted semen and plain yolk-citrate diluter stored for 0, 8 and 16 days.

The results of the bacterial plate counts are shown in figures 2, 3 and 4. Logarithmic rather than arithmetic means have been used to express the mean number of bacteria in the nine semen samples. Since 1:10 was the lowest serial dilution employed and at least 25 colonies were required at

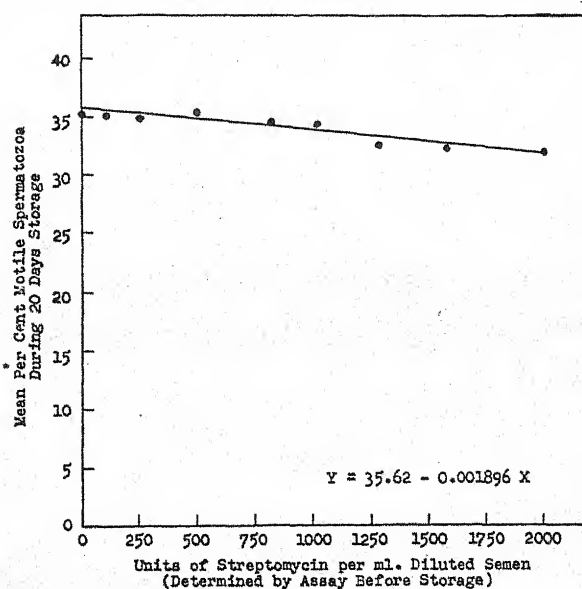


FIG. 1. Relationship of per cent motile spermatozoa during 20 days of storage to level of streptomycin, as shown by regression.

this dilution before a count was considered significant, any counts below log number 2.40 only indicate that the material was not sterile.

As shown in figure 2, levels of streptomycin above 100  $\gamma$  per ml. were most effective in inhibiting growth of bacteria in freshly diluted semen. Complete inhibition was obtained at all levels of streptomycin in seven of the nine samples. Freshly diluted semen without streptomycin contained an average of 5,000 bacteria per ml., as compared to an average of only 120 bacteria per ml. for the portions of diluted semen containing added streptomycin.

Figure 3 shows that all levels of streptomycin retarded bacterial growth in diluted semen stored for 8 days at 4.5° C. The tubes of untreated

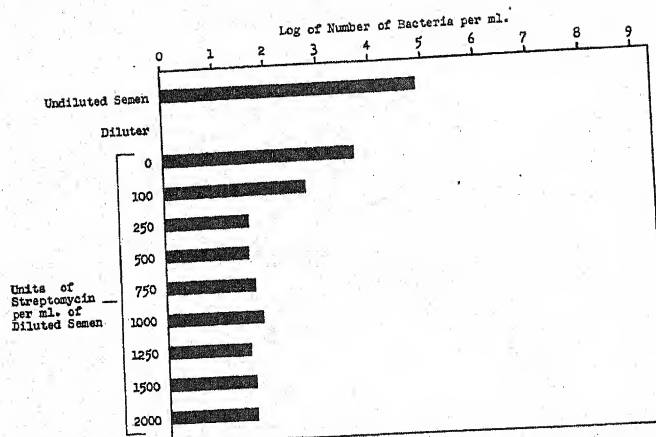


FIG. 2. The effect of streptomycin upon bacterial growth in freshly diluted semen.

semen averaged 82,000 bacteria per ml. while those containing streptomycin averaged 2,000 bacteria per ml. of diluted semen. The high average counts at the 500 and 1,500  $\gamma$  levels were due to one sample of semen. Possible explanations are contamination during bacteriological analysis or contamination of the individual test tubes of diluted semen with streptomycin-resistant organisms when the tubes were opened for routine motility observations during storage.

Culture plate counts made after 16 days of storage, as shown in figure 4, were rather erratic. In four of the nine diluted samples, minute, pin-point colonies were present which made counting rather difficult. However, in the remaining five samples streptomycin showed fairly good inhibition of bacterial growth as compared with the controls. The average bac-

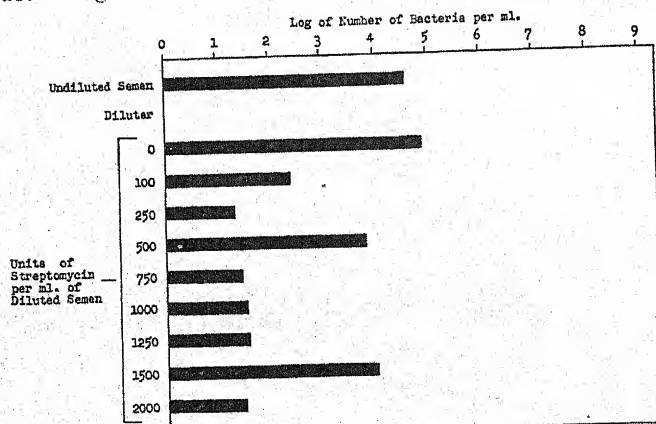


FIG. 3. The effect of streptomycin upon bacterial growth in diluted semen stored for 8 days at 4.5° C.

terial count for diluted semen without streptomycin was 131,000,000 per ml., while the average for diluted semen containing the various levels of the antibiotic was 137,000 per ml. Thus, the antibacterial activity of streptomycin was greatest in freshly diluted semen and semen stored for 8 days.

Very few typical bacteria of the coliform group were present on the desoxycholate agar plates. Only one sample had countable plates and these were present only in the undiluted semen and the tube of diluted semen which did not receive streptomycin. The number of bacteria of the coliform group increased in these two tubes during storage. While there were only a few organisms of this type per ml. in the fresh undiluted semen, counts of 250 and 50,000 per ml. were obtained after storage for 8

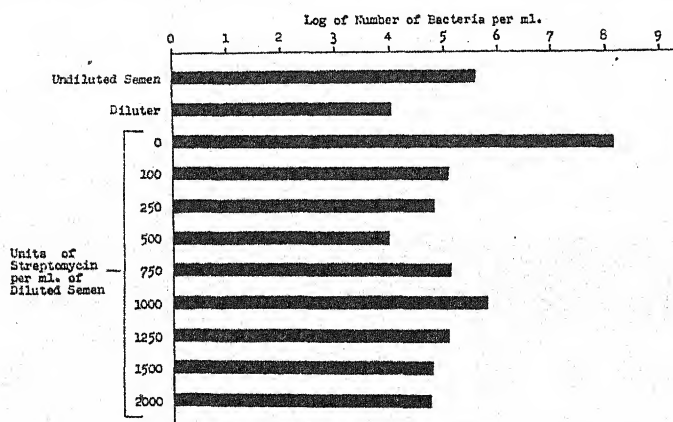


FIG. 4. The effect of streptomycin upon bacterial growth in diluted semen stored for 16 days at 4.5° C.

and 16 days, respectively. The number of coliform bacteria in the untreated diluted semen increased from a few colonies before storage to 5,000 per ml. after 8 days of storage and 1,000,000 per ml. after 16 days of storage.

Total plate counts on the ten samples of fresh, undiluted semen used in the final livability study ranged from 2,000 to 350,000 bacteria per ml., with a mean of 73,000 bacteria per ml. The samples were collected with an artificial vagina and only two of the ten ejaculates had counts exceeding 100,000 bacteria per ml., while seven of the remaining eight had counts of 30,000 or less per ml.

*Stability of streptomycin in diluted semen.* The stability of streptomycin in diluted semen stored at 4.5° C. was determined by assays at 0, 8 and 16 days with the standard cylinder plate method, using *Bacillus subtilis* as the test organism. The results of the assays made on ten diluted semen samples are presented in table 2. There was no appreciable decrease in the amount of streptomycin over the 16-day storage period.



Studies are now in progress to test the effect of streptomycin upon the fertility of diluted semen used for artificial breeding. Its use in combination with penicillin also is being studied and will be reported as soon as the work is completed.

TABLE 2

*The stability of streptomycin in diluted semen stored at 4.5° C.*  
(Mean of 10 determinations)

Theoretical units of streptomycina	Units of streptomycin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
100	96	96	95
250	246	252	247
500	501	520	502
750	814	774	756
1000	1014	1094	988
1250	1282	1250	1267
1500	1586	1511	1529
2000	2012	2065	2014
Diluter alone	0	0	0

<sup>a</sup> No. of units expected, based on the total units in the ampules according to the producer.

## SUMMARY

1. The additions of 100, 250, 500, 750 and 1,000  $\gamma$  of streptomycin per ml. of diluted semen did not significantly affect the livability of bull spermatozoa during a 20-day storage period. Levels of 1,250, 1,500 and 2,000  $\gamma$  per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a storage period of 20 days.

2. A significant linear relationship was found between spermatozoan livability and concentration of streptomycin. The mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250  $\gamma$  of streptomycin.

3. Streptomycin inhibited bacterial growth in diluted semen as compared with untreated controls. Levels above 100  $\gamma$  per ml. were especially effective; the greatest antibacterial activity was obtained in freshly diluted semen and diluted semen stored for 8 days. The initial plate counts for ten ejaculates studied ranged from 2,000 to 350,000 bacteria per ml. of fresh, undiluted semen, with a mean of 73,000 bacteria per ml.

4. There was no significant loss in streptomycin activity in diluted semen stored for 8 and 16 days at 4.5° C.

## REFERENCES

- (1) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Glycolysis, and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 11-19. 1948.

- (2) GUNSALUS, I. C., CAMPBELL, J. J. R., BECK, G. H., AND SALISBURY, G. W. The Bacteriology of Bull Semen. II. The Effect of Bacteria upon Rapid Tests for Semen Quality. *J. Dairy Sci.*, 27: 357-364. 1944.
- (3) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. *J. Dairy Sci.*, 24: 911-919. 1941.
- (4) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (5) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (6) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (7) WAKSMAN, S. A., AND REILLY, H. C. Bactericidal Action of Antibiotic Substances. *J. Infectious Diseases*, 75: 150-159. 1944.





## EFFECT OF ULTRAVIOLET IRRADIATION ON BACTERIOPHAGE ACTIVE AGAINST *STREPTOCOCCUS LACTIS*<sup>1</sup>

G. I. GREENE<sup>2</sup> AND F. J. BABEL<sup>3</sup>

Iowa Agricultural Experiment Station, Ames

Various methods have been advocated for decreasing the incidence of slow acid production due to bacteriophage action during the manufacture of cheese. The control measures have included protection of the mother culture, bulk culture and cheese milk from bacteriophage, and also methods for the destruction of bacteriophage within the cheese plant. Chlorination and irradiation with ultraviolet light commonly have been employed for the destruction of bacteriophage. Since bacteriophage particles frequently are found in the air of the cheese plant, mists containing active chlorine have been used for their destruction. Chlorine mists have the disadvantage of corroding equipment and fixtures within the plant. Use of ultraviolet irradiation for the destruction of bacteriophage would possess various advantages over the use of chlorine compounds, provided it was as effective.

### HISTORICAL

Appelmans (2) and Zoeller (10) found that *Shigella* bacteriophage was killed by a short exposure to ultraviolet rays. Mizuno (7) noted that the depth of solution containing bacteriophage, type of suspending liquid, and concentration of bacteriophage influenced the time required for destruction of *Shigella* bacteriophage by ultraviolet rays.

Gates (3) exposed a culture of *Staphylococcus aureus* and its homologous bacteriophage to ultraviolet light and noted a direct relation between the energy required to kill the organism and that needed for inactivation of the bacteriophage; bacteriophage required expenditure of more energy for its destruction.

Sutton (8) exposed various quantities of a bacteria-free filtrate containing bacteriophage active against *Streptococcus cremoris* to ultraviolet rays. When 2-, 4-, and 9-ml. quantities of the filtrate were placed in petri dishes at a distance of 3 inches from a Westinghouse Sterilamp, the bacteriophage was destroyed completely in 6 minutes.

Received for publication February 13, 1948.

<sup>1</sup> Journal Paper J-1520 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 652.

<sup>2</sup> A portion of a thesis presented in partial fulfillment of the requirements for the degree of Master of Science by the senior author. Present address: Pet Milk Co., Greenville, Tennessee.

<sup>3</sup> Present address: Dairy Husbandry Department, Purdue University, Lafayette, Indiana.

Luria and Delbrück (5) stated that a suspension of bacterial virus, after inactivation by ultraviolet rays, may have the ability to interfere with growth of a second virus acting on the same host, *Escherichia coli* in this case. A virus inactivated by ultraviolet irradiation inhibited the growth of sensitive organisms but did not lyse bacterial cells.

Anderson (1) conducted experiments to determine whether cells of *E. coli* which had been inactivated by ultraviolet irradiation could be used as hosts for the propagation of bacterial virus. Bacterial cells which were not able to form colonies were able to support growth of virus. Irradiation appeared to reduce the ability of the host to adsorb virus, liberate a virus-inhibiting substance from the host, reduce burst size of the host, inactivate the virus when adsorbed on the host, and kill bacteria.

Whitehead and Hunter (9) stated that bacteriophage active against lactic streptococci could be destroyed by ultraviolet light if the necessary exposure was given. The practical value of ultraviolet light for the destruction of bacteriophage within a cheese plant was considered questionable because of constant reinfection.

Latarjet and Wahl (4) noted that bacteriophage preparations and homologous strains of *E. coli* irradiated separately were not destroyed in the same length of time. Bacteriophage was two to six times more sensitive to ultraviolet irradiation than the homologous strain of *E. coli*. However, when a mixture of bacteriophage and cells was irradiated, the bacteriophage was more resistant.

Luria and Latarjet (6) state that *E. coli* loses its ability to liberate bacteriophage after irradiation, due to inactivation of the intracellular bacteriophage. When bacteria were irradiated between the time of infection with bacteriophage and lysis of the organisms, a rapid increase in resistance was noted. The increase in resistance was thought to be caused by an accumulation of ultraviolet-absorbing materials around the bacteriophage particle. Analysis of the survival curves for bacteriophage indicated that more than one bacteriophage particle grew in one host cell.

#### METHODS

*Source of ultraviolet light.* The sources of ultraviolet light were commercial low-pressure mercury-vapor lamps, releasing 85 per cent of their radiations in the range of 2537 Å. One bulb had an output of 279 microwatts and the other bulb an output of 364 microwatts at the surface of the light source.

*Determination of effective radiant energy.* Measurement of the effective radiant energy of the ultraviolet lamps was made with a Luckiesh-Taylor germicidal light filter in combination with a standard General Electric light meter.

*Preparation of bacteria-free filtrates.* The bacteria-free filtrates were prepared by adding 1 ml. of a sensitive culture of *Streptococcus lactis* to 100 ml. of sterile skim milk. Bottles containing the sensitive culture were placed in an incubator at 30° C. for 3 hours; then 1 ml. of bacteriophage active against the *S. lactis* culture was added. The bottles containing bacteriophage and a sensitive strain of *S. lactis* were incubated at 30° C. for 48 hours. After incubation, the bottle contents were coagulated with sterile 10 per cent lactic acid, filtered through coarse filter paper, and the resulting filtrate passed through a Selas microporous filter of 03 porosity.

*Determination of bacteriophage titer of bacteria-free filtrates.* The serial dilution method was used to determine the concentration of bacteriophage in a bacteria-free filtrate. The bacteriophage titer was recorded as the smallest amount of bacteria-free filtrate, in milliliters, which would cause a significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk by a sensitive culture of *S. lactis*.

*Irradiation of bacteria-free filtrates containing bacteriophage.* A pure culture of *S. lactis* (H1-1) and its homologous bacteriophage (H1-7) were used throughout this series of experiments. The ultraviolet lamps used in the studies were permitted to burn for a period before use in order to stabilize the radiant energy output.

Irradiation experiments were carried out by placing the desired quantity of bacteria-free filtrate containing bacteriophage in a petri dish, distributing the filtrate evenly over the bottom surface and irradiating with the cover removed from the petri dish. Petri dishes having a flat bottom surface were selected for use; they had average inside diameters of 90 mm.

After a bacteria-free filtrate containing bacteriophage was irradiated for a given time, a portion of the filtrate was added to tubes of litmus milk which had been inoculated just previously with a sensitive culture. A significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk, as compared with the control cultures, denoted the presence of active bacteriophage.

#### RESULTS

*Irradiation of 1-ml. quantities of bacteria-free filtrates containing bacteriophage.* One-milliliter quantities of bacteria-free filtrates, having bacteriophage titers of  $10^{-3}$ ,  $10^{-6}$ , and  $10^{-11}$ , were irradiated with two commercial low-pressure, mercury-vapor ultraviolet bulbs. With each bulb, 1-ml. portions of the bacteria-free filtrates were irradiated at distances of 3, 6, 9, 12, 18 and 24 inches from the source of light. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 1.



The data show that bacteriophage in a bacteria-free filtrate having a titer of  $10^{-3}$  was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of  $10^{-6}$ , when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb. Also, a bacteria-free filtrate having a bacteriophage titer of  $10^{-8}$  was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of  $10^{-11}$  when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb.

The time necessary for destruction of bacteriophage increased as the distance between the ultraviolet bulb and the bacteria-free filtrates containing bacteriophage was increased. This relationship was noted with filtrates having different bacteriophage concentrations and with both ultraviolet lamps.

TABLE I  
*Inactivation times during irradiation of 1-ml. quantities of bacteria-free filtrates containing various concentrations of bacteriophage*

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
$10^{-3}$	279	7.5	7.5	10	20	45	90
$10^{-3}$	364	5.0	7.5	7.5	10	25	35
$10^{-6}$	279	15.0	30.0	45	75	150	210
$10^{-6}$	364	7.5	15.0	30	45	75	120
$10^{-11}$	279	120.0	150.0	270	360	420	900
$10^{-11}$	364	90.0	120.0	210	300	330	420

The output of radiant energy by an ultraviolet bulb influenced the time required to destroy bacteriophage in a bacteria-free filtrate. Bacteriophage in a bacteria-free filtrate was destroyed in a shorter time by an ultraviolet bulb having an output of 364 microwatts than it was by a bulb having an output of 279 microwatts, when comparisons were made at the same irradiation distance and using filtrates of the same bacteriophage titer.

*Irradiation of 2.5 mm. depths of bacteria-free filtrates containing bacteriophage.* Quantities of bacteria-free filtrates sufficient to form a layer 2.5 mm. deep in petri dishes and having bacteriophage titers of  $10^{-3}$ ,  $10^{-7}$  and  $10^{-10}$  were irradiated with the two ultraviolet bulbs described previously. The irradiation distances were the same as those used for the irradiation of 1-ml. quantities of filtrates. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 2.

The data show that the time necessary for destruction of bacteriophage in bacteria-free filtrates by ultraviolet light was dependent upon the titer of the filtrate. Bacteriophage in a filtrate having a titer of  $10^{-3}$  was destroyed in a shorter time than bacteriophage in filtrates having titers of  $10^{-7}$  or  $10^{-10}$ , when comparisons were made at the same irradiation distance and with the same ultraviolet bulb. Under the same conditions, bacteriophage in a filtrate having a titer of  $10^{-7}$  was destroyed in a shorter time than bacteriophage in a filtrate having a titer of  $10^{-10}$ .

As in the previous experiment, the time necessary for destruction of bacteriophage by ultraviolet light was increased by increasing the irradiation distance. Also, the output of radiant energy by the ultraviolet bulb influenced the time required to destroy bacteriophage in bacteria-free filtrates; the bulb having the greater energy output destroyed bacteriophage in a shorter time under the same conditions.

TABLE 2

*Inactivation times during irradiation of 2.5-mm. films of bacteria-free filtrates containing various concentrations of bacteriophage*

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
$10^{-3}$	279	21	26	39	60	70	80
$10^{-3}$	364	16	20	35	45	55	70
$10^{-7}$	279	40	60	75	90	90	115
$10^{-7}$	364	35	45	60	60	75	105
$10^{-10}$	279	120	135	180	210	225	255
$10^{-10}$	364	60	75	90	135	180	225

*Comparison of irradiation times necessary to destroy bacteriophage in thin and thick films.* The data presented in table 1 show that there were only slight differences in the time required for destruction of bacteriophage in a bacteria-free filtrate having a titer of  $10^{-3}$  by two ultraviolet lamps having different energy outputs at irradiation distances of 3, 6 and 9 inches. With irradiation distances of 12 inches or more, there were greater variations in destruction time between the two lamps. The same general relationship was noted with this filtrate (titer  $10^{-3}$ ) in table 2.

A comparison of the results obtained with bacteria-free filtrates having bacteriophage titers of  $10^{-3}$  shows that at irradiation distances of 3, 6, 9, 12 and 18 inches, bacteriophage in 1-ml. quantities (thin film) was destroyed in a shorter time than bacteriophage in films 2.5 mm. thick. Similar results were obtained with both lamps. At an irradiation distance of 24 inches, the filtrate having a bacteriophage titer of  $10^{-3}$  was destroyed in less time in a 2.5 mm. depth with one lamp (279 microwatts output) than





## ABSTRACTS OF LITERATURE

### BOOK REVIEWS

1. **Henrici's molds, yeasts, and actinomycetes.** 2nd Ed. C. E. SKINNER, C. W. EMMONS, AND H. M. TSUCHIYA. 409 pp. \$5.00 John Wiley and Sons, Inc., New York, N. Y. 1947.

The authors have completely rewritten and considerably expanded the original edition, written in 1930. Their efforts have been directed toward the preparation of a book on yeasts, molds, and actinomycetes chiefly for students of bacteriology. In addition to expansion of previous chapters, new chapters have been included on variations in the lower fungi, infections caused by molds, pathogenic yeast-like fungi, and antibiotic substances. The revisions in classification are in some respects quite radical. Some of the suggestions undoubtedly will meet the approval of most mycologists, but others probably will meet considerable opposition. Examples of proposed changes in generic and species designation for some common dairy microorganisms are: *Geotrichum candidum* for the mold now known as *Oospora lactis* or *Oidium lactis*, *Cryptococcus sphaerica* for the yeast known as *Torula sphaerica*, *Scopulariopsis brevicaulis* for *Penicillium brevicaulis*.

The treatment of the various individual species of molds, yeasts, and actinomycetes is necessarily brief. In view of the present lack of any adequate source of information on many such organisms and their increasing importance in recent years, it is hoped that in future editions the authors will expand their descriptions and discussions in this field.

P.R.E.

2. **Practical emulsions.** 2nd Ed. H. BENNETT. 568 pp. \$8.50. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1947.

After a general discussion of emulsions and emulsifying agents, specific technical applications are discussed by the author and by others, whose papers are presented in the form of a symposium on emulsifying agents and emulsions in industry. Lists of commercial surface-active agents, emulsifying agents, emulsions, and demulsifying and defoaming agents are given, with references to the original literature. A long section is concerned with formulas for emulsions for specific purposes. The sections concerned with food contain little of direct applicability to the dairy industry. F.E.N.

3. **The chemical composition of foods.** 2nd Ed. R. A. McCANCE AND E. M. WIDDOWSON. 156 pp. \$3.75. Chemical Publishing Co., Inc., New York, N. Y. 1947.

The data presented are the results of studies carried out by the authors

and their associates in Great Britain over a considerable period of time. The data are presented in two series of tables, one based upon 100-g. samples and one on 1-oz. samples, and recipes for the cooked foods analyzed are given in a separate section. Data on vitamin content are not given. Water, sugar, starch and dextrins, total nitrogen, protein, fat, available carbohydrate, 9 minerals, calorie value, and "acid-base balance" are given for each food. The various foods are arranged in groups, one of which consists of dairy products.

F.E.N.

4. **Food products.** SAUL BLUMENTHAL. 986 pp. \$12.00. Chemical Publishing Co., Inc., New York, N. Y. 1947.

This book is a compendium of formulas and procedures for the manufacture of a great variety of food products. A chapter is devoted to milk and milk products, and these materials are mentioned in a number of other places. Although the book contains a considerable amount of information, numerous errors are apparent, some of the material definitely is out of date and the treatment is spotty. Pages are devoted to a particular process, patent or minor product, whereas a topic as important as pasteurization of milk is dismissed with only cursory remarks and practically no specific information, and the same is true of a number of other important dairy products. A 5% DDT solution is recommended for insect control, but no information concerning formulation or specific methods of application is given. The sections of microbiology and the relationship of microorganisms to sanitation are very incomplete. Many of the references given in the brief bibliography found in the appendix have been superseded by more recent material.

F.E.N.

## BACTERIOLOGY

5. **Observations on the use of a modified direct microscopic method for estimating bacterial quality of raw and pasteurized milk.** G. W. WATROUS, JR., AND F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College. *J. Milk and Food Technol.*, 10, 5: 269-275. Sept.-Oct., 1947.

A modification of Newman Lampert formula no. 2 was recommended by using only 20% of the methylene blue dye and 20% of the glacial acetic acid as originally recommended. This stain gives a clear, light blue background, free of debris, with bacteria retaining the stain so tenaciously that identification easily was accomplished.

A ratio of one to one was attained with the modified stain and Levowitz's strip-counting technic on samples of raw milk when the plate and direct microscopic count were compared. There is no appreciable relationship



between this microscopic clump count and the plate count of organisms in fresh, laboratory-pasteurized milk. Non-viable organisms are capable of stain retention to a varying degree, depending on the microflora of the milk. Microscopic clump counts on fresh laboratory-pasteurized milk are lower than those prior to pasteurization, indicating that all the cells destroyed by pasteurization are capable of staining. A correlation exists between a high microscopic count in raw milk and the same milk after pasteurization. This relationship is not so striking where the bacterial content is relatively low in the raw and pasteurized milk. H.H.W.

6. El B.C.G. en el Uruguay. Catorce años de experiencia sobre pasajes continuados en papa-bilis-glicerina. (The B.C.G. in Uruguay. Fourteen years of experience with continuous passage on potato-bile-glycerine medium.) A. TORTORELLA. Rev. med. vet., 4, 43: 712-717. May, 1947.

A summary of a series of experiments that have been carried out on the B.C.G. bacillus for 14 yr. is given. The B.C.G. is not a changeable bacillus and therefore may be considered a definite strain. The successive passage of the organism in bile-potato-glycerine medium influenced neither the vitality of the bacillum nor its antigenic properties. The vaccine prepared from the B.C.G. strain, which had been grown and transferred in bile-potato-glycerine medium, showed a definite activity which was reflected in the following facts: (a) It grew abundantly when cultured; (b) it produced characteristic lesions in experimental animals, but these lesions were not progressive and the animals lived; (c) it produced positive tuberculin allergy in the guinea pigs used for the experiment, even 1 yr. after the inoculation; (d) it formed specific antibodies in the blood serum of experimental rabbits. R.E.M.

7. La accion inhibitoria de la piocianina frente a las *Brucellas abortus*, *suis* y otros agentes. (The inhibitory action of pyocyanin against *Brucella abortus*, *suis* and other agents.) N. PRADINES, Brazil. Rev. med. vet., 4, 43: 718-726. May, 1947.

The organism *Pseudomonas pyocyanea* inhibited the growth of both *Brucella abortus* and *Brucella suis* in culture media. Pyocyanin, a water soluble blue pigment produced by the *Pseudomonas* organism, was found to have, by itself, the same action against the brucellas. This pigment had bacteriostatic and probably bactericidal, but not bacteriolytic, effects against those organisms. When tested *in vitro*, pyocyanin did not alter the phagocytic action of the white blood cells and retained its antibiotic action against the brucella organisms in the presence of blood serum. It also seemed to retain that property when tested *in vivo* in a chicken embryo.



Fluorescein, hemipyocyanin, and the antigen lipid-glucide, other products of *P. pyocyanea*, did not show any antibiotic action *in vitro* against the brucella species under consideration.

Preliminary experiments for a therapeutic value of pyocyanin *in vivo* against *B. abortus* and *B. suis* were carried out. The inoculation of pyocyanin did not cause any disturbances or lesions in small laboratory animals. The pigment easily was eliminated by the urine. Studies along this line still are being carried out and probably will be reported in the future. Pyocyanin also was found to have antibiotic action against *Pasteurella avicide*.  
R.E.M.

8. Growth of *Staphylococcus aureus* in various pastry fillings. W. H. CATHCART, W. J. GODKIN, AND G. BARNETT. The Great A & P Tea Co., New York, N. Y. Food Research, 12, 2: 142-150. March-April, 1947.

Commercial dry-mixed puddings containing milk were found to support the growth of cultures of pathogenic *Staphylococcus aureus* when this organism was inoculated into them and the temperature made favorable. The organism was inhibited when water was substituted for the milk, but the quality of the pudding suffered. Vanilla, pumpkin, squash, and sweet potato pie fillings, cheese cake fillings, and whipping-cream mixes also were found to support the growth of this organism. By using citric acid to lower the pH of vanilla fillings to 3.43-3.65, the growth of the inoculant was checked but the filling acquired a sour taste. A better-tasting cheese cake filling was had by using lactic acid in place of citric; the staphylococcus was effectively inhibited at pH 4.42 to 4.67.  
F.J.D.

#### BUTTER

9. Studies on the neutralization of cream for buttermaking. VII. Neutralization in practice in the butter-factory. F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerton North. New Zealand J. Sci. Technol., 28A, 2: 132-138. Aug., 1946.

The amounts of sodium bicarbonate needed to neutralize creams of different acidities are presented in a chart and techniques employed during neutralization are described.  
W.C.F.

10. Control of moisture content of butter during butter manufacture. F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 1: 31-37. June, 1946.

The formula for calculation of the amount of water to be added to

partially-worked butter in a churn to bring the water content to a definite limit is as follows:

$$\text{Quantity to be added} = \frac{\text{desired H}_2\text{O} - \text{actual H}_2\text{O}}{\text{content (\%)} \quad \text{content (\%)}} \times \frac{\text{estimated load of butter in churn}}{100 - \text{actual H}_2\text{O content (\%)}} \times \frac{1}{1}$$

A chart calculated on the basis of this formula is presented; it is not applicable for calculation of salt to be added. Factors affecting the amount of water to be added are discussed.

W.C.F.

11. Land-cress taint in cream and butter. Parts I and II. F. H. McDOWALL, I. D. MORTON, AND A. K. R. McDOWELL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 5: 305-315. Feb. 1947.

Land-cress (*Coronopus didymus*) is an annual weed, of the family *Cruciferae*, which appears in young pastures and in bare patches in old pastures. When consumed by cows, it imparts an off flavor to milk and cream, butter, and cheese derived from it. Both land-cress and garden-cress contain a mustard-oil glucoside and both yield benzyl cyanide on direct steam distillation and benzyl isothiocyanate by the silver salt method. The distillate of land-cress also has a peculiar burnt odor.

W.C.F.

12. Land-cress taint in cream and butter. Part III. Relation of conditions of feeding land-cress to cows to incidence of land-cress taint in cream and butter. F. H. McDOWALL, I. D. MORTON, AND J. J. O'DEA, Dairy Research Inst. (N. Z.), Palmerston North, AND A. V. ALLO, Dept. of Agr., Tauranga. New Zealand J. Sci. Technol., 28A, 6: 370-384. April, 1947.

Consumption of as little as 2 oz. of cress by cows caused taint in the cream from milk drawn within 30 min. from the time of eating. When cows ate the cress at noon, the taint was strong in the cream of milk drawn 9 hr. later and was present in milk drawn the following morning. Thus, removal of cows from pastures containing the cress did not prevent the taint in the following milkings but did cause a decrease in the intensity of the defect. Land-cress hay caused a weak taint in the cream. Measures are described for the prevention of the growth of the cress in pastures. If the weed is present, only dry stock should be pastured there.

W.C.F.

13. The washing of butter and its effect on curd content and quality. E. G. PONT, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 432-437. Nov., 1946.

See Abs. 279, J. Dairy Sci., 30, 9: A122.



## CHEESE

14. A practical system for determining the premium value for high fat Cheddar cheese. A. B. EREKSON, Plymouth, Wis. *Natl. Butter Cheese J.*, 38, 9: 44. Sept., 1947.

This discussion is a development of a previous article. (See Abs. 311, *J. Dairy Sci.*, 29: A144. 1946.) It shows that when a working standard of 52.5% fat in the dry matter of the cheese is adopted, higher amounts of fat command a premium and lower amounts a deduction from the market price of the cheese. The value of the fat is obtained by: (a) multiplying the price per pound of 92 score butter by 1.15 to get the approximate value of fat in cream; and (b) deducting the value of 1 lb. of cheese solids. This latter value is calculated by dividing the price of cheese by 0.61, the minimum amount of dry matter permitted by law in a lb. of Cheddar cheese. A table showing the value of fat for numerous butter and cheese price combinations is given.

W.V.P.

15. Factors influencing the texture of Cheddar cheese. G. H. WILSTER, Oregon State College, Corvallis. *Natl. Butter Cheese J.*, 38, 10: 48. Oct., 1947.

The texture of cheese is improved by using high-grade milk, even though the milk is pasteurized before making the cheese. Other essentials for cheese of the best texture are: clean, active starter, efficient pasteurization, clean equipment, acidity control, moisture control, and control of temperatures and pressures during cheddaring and pressing of the curd. These means for improving and controlling texture are suggested for treatments of specific defects, including gassy, greasy, open and crumbly texture, and corky and mealy-pasty body and texture.

W.V.P.

16. Pasteurização a jato direto. (Direct steam pasteurization.) J. A. RIBEIRO. *Boletim do leite*, 1 (4ª Epoca), 1: 9-14. July, 1947.

A direct steam pasteurization process to be employed for milk that will be used in the manufacture of cheese is described in detail and a few drawings are given to illustrate it. The advantages credited to this process are: (a) It is a cheap form of pasteurization. (b) It is easy to use. (c) It uses a limited space in the cheese plant. (d) It takes advantage of the vacuum created by the steam injection to raise the milk to higher levels, saving time and human effort. Studies have not been made of the comparative bacteriological efficiency of the procedure.

R.E.M.

17. Fermentos para queijos. O fermento láctico é a alma do queijo. O emprego de fermento perfeito é a base da obtenção de queijos



ótimos. (Ferments for cheese. The lactic ferment is the foundation of the cheese. The use of the perfect ferment is the base in the obtention of optimum cheeses.) J. A. RIBEIRO. Boletim do leite, 1, 9: 1-4, 18. June, 1947.

A brief and general discussion of the different organisms that influence the ripening of the different types of cheeses is given. A recognized method for the day-to-day preparation of the starter, for the manufacture of semi-hard cheeses from pasteurized milk, is presented in detail. The aseptic handling of the cultures throughout the preparation of the starter is emphasized and the beneficial effects of the use of starter in the manufacture of semi-hard cheeses from pasteurized milk are enumerated. R.E.M.

18. Temperature and humidity control in cheese-curing rooms. T. R. VERNON, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 6: 361-369. April, 1947.

Cheese was of better quality after storage in an insulated room controlled automatically at 55° F. and a humidity of 85% than after storage in an uninsulated room or an insulated room without such controls. Some trouble with mold growth was encountered in the controlled room, but weight losses were less. W.C.F.

19. Control measures against the cheese-mites, *Tyrolichus casei* Ouds. and *Tyrophagus longior* Gerv. J. MUGGERIDGE, Plant Research Bureau, Nelson, AND R. M. DOLBY, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 1: 1-30. June, 1946.

Wax protected cheese somewhat against mites, but they were able to bore through the wax layer and enter the cheese. Dusts were ineffective against attacks by mites, and the high humidity necessary in cheese warehouses made the use of dusts difficult. Three fumigants, ammonia, methyl bromide, and dichloroethyl ether, were found effective, with the latter considered the most suitable. It is effective in low concentrations, is easily handled and applied, and is cheap. The chemical may be used in the vapor state as a fumigant, but the authors recommend that it be applied as a liquid to the shelves in the curing room at the rate of 1 lb. per 1,000 cu. ft. of room space or per 100 sq. ft. of shelving. Another method is to place the cheeses on boards treated with the chemical. Methyl bromide is expensive and is not as persistent as dichloroethyl ether. Ammonia is too readily absorbed by surrounding materials. W.C.F.

20. Cheese and its relation to disease. CURRENT COMMENT. J. Am. Med. Assoc., 135, 11: 718. Nov. 15, 1947.

This is a detailed review of the paper of the same title by F. W. Fabian, Am. J. Pub. Health, 37: 987. Aug., 1947.

## CHEMISTRY

21. The thiamin, riboflavin and niacin content of some New Zealand milks. F. H. McDOWALL, Dairy Res. Inst. (N. Z.), N. O. Bathurst, Plant Chem. Laboratory, AND I. L. CAMPBELL, Dairy Research Inst., Palmerston North. New Zealand J. Sci. Technol., 28A, 5: 316-328. Feb., 1947.

Thiamin was estimated by the fluorimetric method and niacin and riboflavin were measured by microbiological methods. The thiamin and riboflavin contents of Jersey milks regularly were higher than those of Friesian milks, but the niacin content was the same for both breeds. The feeding of concentrates to cows on pasture caused a rise in the thiamin content of milk from Jersey cows but not from Friesian cows. W.C.F.

22. The polarographic estimation of ascorbic acid in milk. DAWN R. PERRIN, Dairy Laboratory, Wallaceville, AND D. D. PERRIN, Animal Research Sta., Wallaceville, New Zealand Dept. of Agr. New Zealand J. Sci. Technol., 28A, 4: 266-272. Dec., 1946.

The technique was employed for the estimation of ascorbic acid in milk but it also could be used to measure dehydro-ascorbic acid. W.C.F.

23. The estimation of copper in cream. G. C. DEATH, F. RUTH LIGHT-FOOT, AND G. M. MOIR, Dairy Div. Laboratory, Dept. of Agr., Wallaceville. New Zealand J. Sci. Technol., 28A, 4: 273-284. Dec., 1946.

This is a modification of the filtration method used with butter. Improvements in the wet-ashing method also are described. W.C.F.

24. Determination of iron in foods and food products. W. D. POHLE, J. H. COOK, AND V. C. MEHLENBACHER. Research Laboratories, Swift and Co., Chicago, Ill. Food Research, 12, 3: 229-238. May-June, 1947.

A simple, accurate colorimetric method is described for the determination of iron in food products employing dry ashing and color development with 1, 10-phenanthroline. Phosphates and copper in normal quantities did not interfere. The method was applied to milk and dried milk satisfactorily. F.J.D.

25. Note on the effect of phosphatide on the ferric thiocyanate method of estimating peroxide in fats. G. L. HILLS AND R. WILKINSON, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 430-431. Nov., 1946.

See Abs. 287, J. Dairy Sci., 30, 9: A124.

26. The mechanism of the oxidant effects of commercial salt and water in butterfat. G. L. HILLS AND J. CONOCHIE, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 414-429. Nov., 1946.

See Abs. 286, J. Dairy Sci., 30, A124.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

27. A rapid method for the estimation of whey proteins as an indication of baking quality of non-fat dry milk solids. H. A. HARLAND AND U. S. ASHWORTH, Agr. Expt. Sta., Pullman, Wash. Food Research, 12, 3: 247-251. May-June, 1947.

A method for estimating the baking quality of milk proteins in the form of dry milk, concentrated milk, and fluid milk is presented. The presence of a low concentration of whey proteins (indicating relatively high heat treatment) is shown to correlate with good baking quality. The whey proteins are determined, after the casein of the sample has been salted out, by acidifying the diluted filtrate and measuring the turbidity by means of a colorimeter.

F.J.D.

28. The utilization of buttermilk in New Zealand. F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 2: 97-132. Aug., 1946.

In 1940-1944, buttermilk from New Zealand creameries was used almost exclusively for animal feeding. Variations in the composition of buttermilk are discussed and other uses for the product are described. W.C.F.

29. Directions for making kefir fermented milk. L. A. BURKEY, Bureau of Dairy Ind., U. S. Dept. Agr. BDIM-Inf.-58. 4 pp. Nov., 1947.

Concise directions for handling kefir grains, for preparation of the milk and for handling of the product are given for both small and large scale manufacture.

F.E.N.

### FEEDS AND FEEDING

30. The effect of the ingestion of high levels of riboflavin on the amount in the milk and urine. P. B. PEARSON AND B. S. SCHWEIGERT, Agr. and Mech. College of Texas, College Station. J. Nutrition, 34, 4: 443-453. Oct. 10, 1947.

Riboflavin values of normal goat and sheep milk as measured microbiologically and fluorimetrically agreed closely. After feeding 2 g. of synthetic riboflavin, the microbiological procedure showed an increase in the



riboflavin content of the milk of about 26%. However, the fluorimetric procedure gave values for the milk of about 5 times greater than the microbiological technique. Growth responses of rats fed the milk agreed with the microbiologically determined values rather than the fluorimetric values.

The microbiological and fluorimetric values for riboflavin content of urine of rats fed large amounts (10 or 20 mg.) of riboflavin agreed closely. Both techniques gave similar values for urine from humans fed 20 mg. of riboflavin per day. Goats and sheep fed large amounts of riboflavin evidently metabolize some of the vitamin into a fluorescent compound which is biologically inactive.

R.K.W.

31. Vitamin A intake in cattle in relation to hepatic stores and blood levels. P. H. FREY, R. JENSON, AND W. E. CONNELL. Colorado Agr. Expt. Sta., Ft. Collins. *J. Nutrition*, 34, 4: 421-430. Oct. 10, 1947.

Hereford steers of about 8 months of age were fed a carotene-free basal diet plus vitamin A supplements of 0, 25, 200, and 500 I.U. daily per lb. of body weight. Serum levels of vitamin A and carotene were determined at 0, 27, 83, 159, and 277 days and hepatic values at 0, 166, and 280 days. Animals receiving vitamin A supplements all grew at about the same rate. Dietary vitamin A did not exert a sparing action on hepatic stores of carotene. Vitamin A stores of the liver increased in practically a linear relationship with increased intake. Serum levels of vitamin A increased rapidly up to a daily intake of 100 I.U. per lb. of body weight, when the serum contained about 50 m $\mu$  per 100 ml. at 280 days.

R.K.W.

### FOOD VALUE OF DAIRY PRODUCTS

32. Some studies on the nutritive value of butter fatty acids. E. HEFTMANN, Univ. of Rochester. *J. Nutrition*, 34, 4: 455-467. Oct. 10, 1947.

No significant difference in gain of weights or efficiency of food utilization was found between two groups of young rats (5 males and 5 females in each group) when one group received butter fatty acids and the other group received the non-volatile fatty acids of butter for a feeding period of 5 weeks. Gains of weight and efficiency of food utilization were decreased only in the male rats fed hydrogenated fatty acids when one group received the butter fatty acids and the other group received partially hydrogenated fatty acids; intakes of vitamin A and D were low. In still another trial, the amounts and concentrations of vitamin A stored in the livers of rats fed preformed vitamin A were not significantly affected by complete hydrogenation of the butter fatty acids.

R.K.W.

## HERD MANAGEMENT

33. The influence of certain milking-machine adjustments on the rate of machine-milking. W. G. WHITTLESTON AND S. A. VERRALL, Animal Research Sta., Dept. of Agr., Ruakura. New Zealand J. Sci. Technol., 28A, 6: 406-416. April, 1947.

Variations in pulsator speeds (21, 42, and 84 pulsations per min.) and in vacuums (10, 14.75, and 19 in.) did not cause a significant difference in the milking rate. W.C.F.

34. Milking apparatus. L. F. BENDER AND J. A. SCHMITT. (Assigned to Universal Milking Machine Co.) U. S. Patent 2,429,983, Nov. 4, 1947 (14 claims). Official Gaz. U. S. Pat. Office, 604, 1: 85. 1947.

A teat cup claw is described, the bottom of which easily may be opened for inspection. Suitable connections are provided for collecting the milk from the 4 teat cups and delivering it through a hose to a milk-collecting device. Nipples also are provided for the air which operates the teat cups alternately. R.W.

35. Comparative rates of production of different breeds of dairy cattle. E. G. MISNER. Holstein-Friesian World, 44, 21: 2696-2697. Nov. 1, 1947.

A summary of 1,616 sires proved in D.H.I.A. work in 1945 shows that their daughters, arranged by breeds, averaged 178 lb. less milk, 2 lb. less fat, \$5 less energy value of product, and 0.05 more in fat percentage than their dams. The author interprets these figures to mean that: (a) the sires used are not so good as they should be to maintain the rate of production of our dairy herds or (b) the data are not fair to the bulls. A factor for correctly converting the total fat production to mature total fat production may be too low for converting milk production on young cows and may not give any consideration for decreasing test due to age.

A.R.P.

36. The persistence of DDT on cattle. R. H. HACKMAN, Div. of Ind. Chem. J. Council Sci. Ind. Research (Australia), 20, 1: 56-65. Feb., 1947.

Licking, either by the animal concerned or by another animal, is the most important factor causing the removal of DDT from cattle sprayed with that chemical. The amount of DDT ingested in this way is insufficient to produce toxic symptoms. W.C.F.

37. Stock watering tank. C. FINE. U. S. Patent 2,430,165, Nov. 4, 1947 (3 claims). Official Gaz. U. S. Pat. Office, 604, 1: 131. 1947.

The chief feature of this tank is a tubular chamber spaced above the



bottom, which acts as a fire box for providing heat. The smoke is discharged through a chimney extending upward from the chamber. R.W.

38. Electrical system for cattle stalls. J. J. HANTZ. U. S. Patent 2,428,875, Oct. 14, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 603, 2: 264. 1947.

To improve the sanitary condition of dairy cattle stalls an electrical device is used to cause the cows consistently to deposit the droppings in the gutter back of the stall. The device consists of a horizontal bar, charged with a small electric current, adjustably suspended a few inches over the cow's back. If the cow is not standing in the proper position, she receives a small shock at the time the back is arched prior to evacuation, thereby causing the cow to step backward into position to deposit the droppings into the gutter. R.W.

### MILK

39. The significance of the coliform test in pasteurized milk. P. D. DELAY, California Agr. Expt. Sta., Berkeley. J. Milk and Food Technol., 10, 5: 297-299. Sept.-Oct., 1947.

The coliform test is an aid in detecting faulty post-pasteurization handling. The results of this study indicate that well-equipped plants can, without exceeding the limits of practicability, handle milk which will be less than 1% positive for the coliform test. The present maximum level of less than 5 to 10% is maintained. The ultimate goal is to attain the 1% limit of coliform organisms in pasteurized milk by a gradual process, depending upon existing conditions in a given locality. H.H.W.

40. Agregado de sustancias quimicas a la leche para su conservacion. (Addition of chemical substances to milk for its preservation.) L. J. MURGUIA. Rev. med. vet., 4, 43: 702-711. May, 1947.

Use of hydrogen peroxide, formol, sodium bicarbonate, oxygen, ozone, and trichloronitromethane or "microlysine", chemical substances that have been used to prevent the milk from curdling, was reviewed as a result of the recent use of "microlysine" as a milk preservative in France. Use of any one of the substances under consideration was discouraged and condemned as a backward step in milk sanitation. R.E.M.

### PHYSIOLOGY

41. The characteristics of the milk-ejection curve of normal dairy cows under standard milking conditions. W. G. WHITTLESTON, Ruakura



Animal Research Sta., Dept. of Agr. New Zealand J. Sci. Technol., 28A, 3: 188-205. Oct., 1946.

To ascertain quantitatively what happens when cows are milked mechanically without hand stripping, the milk-ejection curves of a dozen cows were recorded throughout one season by means of a milk-flow recording apparatus. The average rate of milk flow tended to decline toward the end of the season, so that the time of milking did not decrease appreciably. The machine strippings did not increase significantly with declining yield, but the percentage of milk yielded as strippings increased. The starting time tended to increase and become erratic toward the end of lactation. No factor in the strict milking-machine procedure appeared to be harmful to the health of the udder.

W.C.F.

42. Contribucion al estudio del Calcio y Fosforo inorganico en la sangre de la vaca lechera. (Contribution to the study of inorganic calcium and phosphorus of the blood of the milk cow.) F. A. ROJAS. Agricultura tecnica, 7, 1: 20-25. June, 1947.

The amounts of Ca and P in the blood serum of 101 lactating cows, which represented different breeds and different levels of production, were determined. The blood samples were taken before the animals were allowed to eat in the morning. The Ca was found to be present in the range of 8 to 10.8 mg. per 100 cc. of blood serum, with 9.37 mg. as the average. P was present in the range of 2.70 to 7.50 mg. per 100 cc. of blood serum, with 4.77 mg. as the average. The Ca-P ratio fluctuated between 1.70 to 1 and 2.65 to 1, the average being 2.15 to 1. The Ca and P contents found were lower, in general, than those obtained by Haag and Jones in similar experiments.

R.E.M.

## SANITATION AND CLEANSING

43. A study of the germicidal efficiency of can washing compounds. M. J. FOTER AND R. D. FINLEY, Research Laboratory, Pet Milk Co., Greenville, Ill. J. Milk and Food Technol., 10, 5: 257-262. Sept.-Oct., 1947.

The germicidal efficiency of 6 alkaline and 1 acid can-washing compounds was studied in 120 to 130 milk cans. The alkalinity levels were studied within different ranges. The germicidal efficiency was very slight or non-existent at an alkalinity range of 0.05 to 0.10% as  $\text{Na}_2\text{O}$ . When the alkalinity was increased to 0.15 to 0.20% as  $\text{Na}_2\text{O}$ , the germicidal effect was increased markedly with 2 of the cleaners.

When the acid can-washing compound was used, the acidity of the wash solution was neutralized by the calcium and magnesium salts of the hard water. This condition produced an alkaline reaction in the milk cans.

The germicidal efficiency of the acid cleaner was increased if the cleaner was maintained at a point at which the cans were acid.

The authors suggest that careful consideration should be given to the cleansing and germicidal efficiency of washing compounds in addition to rinsability, wetting, emulsification, water conditioning, etc. Germicides are not routinely used in mechanical can washers. Too much dependency should not be placed on the temperature of the rinse water, sterile rinse, temperature of the steam, and hot air for drying. H.H.W.

44. A study of the corrosion of tin plate by can washing compounds.

R. D. FINLEY AND M. J. FOTER, Research Laboratory, Pet Milk Co., Greenville, Ill. *J. Milk and Food Technol.*, 10, 5: 263-268. Sept.-Oct., 1947.

The corrosive action on tin plate was studied by testing 8 commercial alkaline cleaners, 2 commercial acid cleaners, and 4 basic alkalis. All of the commercial alkaline cleaners and basic alkalis readily attacked tin, while the two acid cleaners had very little effect on the tin during the time of exposure. The acid cleaners in some cases showed spots of corrosion, with formation of pits. The corrosive action of sodium hydroxide adjusted to a wide concentration range had very little effect on the tin, except when a very low concentration was used. The oxygen content of the alkali solution probably plays a major rôle in tin corrosion.

The can washing alkalinity range generally used is 0.05 to 0.20%  $\text{Na}_2\text{O}$ , which is equivalent to 0.005 to 0.27%  $\text{NaOH}$ . This strength fell within the sodium hydroxide concentration range where maximum corrosion occurred. Results seem to indicate that a corrosive inhibitor, such as sodium sulfite, should be added to alkaline can washing compounds. An inhibitor would minimize alkaline corrosion of cans and also increase the germicidal action of alkalies in cleaning compounds. H.H.W.

45. The toxicity to houseflies of paints containing DDT. D. GILMOUR, Div. of Econ. Entomol. *J. Council Sci. Ind. Research (Australia)*, 19, 3: 225-232. Aug., 1946.

DDT was added to a glossy enamel, a flat oil paint, an emulsion-type paint, a water paint, and an ordinary oil paint. The most effective mixtures were a glossy enamel containing 20% DDT and a flat oil paint with 3-5% DDT. The effectiveness of the oil paints depended on the degree to which the DDT had crystallized in the film. W.C.F.

## MISCELLANEOUS

46. Pasteurizing apparatus. E. K. KINTNER. (Assigned to Arco Welding and Machine Works.) U. S. Patent 2,428,880, Oct. 14, 1947 (3 claims). Official Gaz. U. S. Pat. Office, 603, 2: 266. 1947.

A pasteurizer of the plate heat-exchanger type is described. A rubber gasket vulcanized to the perimeter of each plate prevents leakage. The equipment is characterized by a centrally located baffle which causes the liquid to flow up one side and down the other between any two plates, the direction being reversed between the next two plates for maximum efficiency of heating or cooling.

R.W.





## ABSTRACTS OF LITERATURE

### BOOK REVIEWS

47. Fatty acids. Their chemistry and physical properties. KLARE S. MARKLEY. 668 pp. Interscience Publishers, Inc., New York, N. Y. 1947.

This volume is divided into six sections as follows: A. The Nature and History of Fats and Waxes; B. Classification and Structure of the Fatty Acids; C. Physical Properties of the Fatty Acids; D. Chemical Reactions of the Fatty Acids; E. Synthesis of Fatty Acids; and F. Isolation and Identification of Fatty Acids. The material is covered in 23 chapters. The treatment is exhaustive and systematic. Numerous references to the original publications are given, and the author index permits ready reference to the publications of any one worker or group of workers. A 20-page subject index greatly improves the value of the book as a reference volume. Numerous figures, tables and chemical formulas are employed to advantage. In the opinion of the reviewer, this volume is a valuable addition to the reference literature.

F.E.N.

48. Annual review of biochemistry. Vol. 16. J. MURRAY LUCK, Editor. 740 pp. \$6.00. Annual Reviews, Inc., Stanford University P.O., Calif. 1947.

This volume continues the standard of excellency set by the preceding publications in the series. Among the 25 chapters, those of particular interest to people in the dairy industry probably are the following: Biological Oxidation and Reduction; Proteolytic Enzymes; The Chemistry of the Carbohydrates; The Chemistry and Metabolism of the Lipids; Phosphorus Compounds; Carbohydrate Metabolism; The Metabolism of Proteins and Amino Acids; Antioxidants; Choline; The Chemistry of the Proteins and Amino Acids; Mineral Metabolism; The Chemistry of the Hormones; Fat-soluble Vitamins; Water-soluble Vitamins; The Use of Pteroylglutamic Acid (Liver *L. casei* Factor, Folic Acid) in Clinical Studies; Nutrition; Carotenoid and Indolic Biochromes of Animals; Bacterial Metabolism; The Use of Isotopes in Biochemical Research: Fundamental Aspects; and The Chemistry of the Steroids. The volume serves admirably as a reference book which may be used easily because of the orderly arrangement of the material presented.

F.E.N.

### CHEESE

49. Sweet curd cottage cheese. N. C. ANGEVINE, Angevine Dairy Laboratory, Springfield, Mo. Milk Dealer, 37, 1: 46, 132-138. Oct., 1947.

Following a brief history of cottage cheese, sweet curd cottage cheese

is defined as a cottage cheese of lower acidity which involves a definite method of manufacture with an actual control of acidity. It must be cut into cubes of equal size. It must be made by the use of a small amount of rennet or, better still, with good commercial cheese coagulator. The proper amount of coagulator is necessary to set up the curd firmly enough that it may be cut in cubes that will hold their shape at a whey acidity of 0.50 to 0.53%. Use of the short method whereby the cheesemaker controls his cheesemaking throughout is necessary. The author then discusses the advantages of the short method, the equipment needed and the short method procedure in detail.

C.J.B.

### CHEMISTRY

50. Determination of high molecular weight quaternary ammonium compounds as the triiodides. O. B. HAGER, E. M. YOUNG, T. L. FLANAGAN, AND H. B. WALKER. Rohm & Haas Co., Philadelphia, Pa. Ind. Eng. Chem., Anal. Ed., 19, 11: 885-888. Nov., 1947.

Two qualitative and three quantitative methods of analytical value are described which are based on the insolubility of the triiodides of many high molecular weight quaternary ammonium compounds in water. These triiodides are precipitated rapidly from aqueous solution, redissolved in dilute alcohol and determined colorimetrically or by titration with sodium thiosulfate or by potentiometric titration with a solution of iodine. One quantitative method is simple enough for the field use of health inspectors testing sanitizing rinse solutions; another is a versatile laboratory potentiometric method.

B.H.W.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

51. Plastic cream—its production and uses. R. J. SPEERS, Abbotts Dairies, Philadelphia, Pa. Natl. Butter Cheese J., 38, 12: 48. Dec., 1947.

Plastic cream, first developed about 1920, now is well established in commerce. It is used largely in ice cream and cream cheese but can be homogenized to make table cream. The baking industry accepts it reluctantly because of its bland flavor. It must be made from raw material of high quality and must be free of iron or copper contamination. The cream is heated to a minimum of 170° F. for 15 min. after the first separation and is held at 145° F. during the second separation. The proper container is a cylindrical cardboard type like a 5-gal. ice cream can. As soon as it is packaged, the cream is frozen in an air blast at -10 to -20° F., held at 0 to -10° F., and is shipped in refrigerated, brine-tank freight cars. A careful manufacturing process makes cream with from 79.5 to 80.5% fat.



Standard plate counts show very few bacteria in the finished product, although work should be done on psychrophilic types. Plastic cream must be carefully made and properly used to maintain the trade advantage it has earned in recent years.

W.V.P.

52. Cream separator. C. M. WICKSTRUM. U. S. Patent 2,431,596, Nov. 25, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 604, 4: 704. 1947.

A conventional type glass milk bottle, containing milk which has been allowed to cream, is capped with an airtight cover, carrying 2 tubes. To the outer end of one is attached a rubber bulb for forcing air into the capped bottle just below the cap. The cream is forced out of the bottle through the second tube, the inner end of which is positioned just above the cream line and the outer end of which is bent downward to facilitate collection of the cream.

R.W.

## DISEASES

53. Brucellosis as an occupational disease. T. B. RICE. J. Am. Vet. Med. Assoc., 111, 849: 470-472. Dec., 1947.

A very high percentage of all veterinarians engaged in large animal practice already have, or have had, brucellosis. The disease easily is contracted by direct contact with infected animals or raw products from infected animals. The type of organism causing abortion in goats and swine gives a more severe reaction in humans than does the cattle strain. All possible precautions should be exercised to prevent the spread of the disease. Veterinarians, technicians and laboratory workers should wear rubber gloves in all contact work with unknown specimens. All foods and milk that are from possibly contaminated animals or areas should be pasteurized or heated before consumption. The germs easily are killed by heat and almost any sort of cooking is sufficient to insure complete safety to consumers. Brucellosis is extremely hard to diagnose in humans and, at the present time, treatment is not very effective. Vaccine treatments have not proved too effective as yet.

T.M.L.

54. Brucella agglutination tests and vaccination against cholera. C. W. EISELE, N. B. McCULLOUGH, GRACE A. BEAL, Dept. of Medicine, School of Medicine, Univ. of Chicago; AND W. ROTTSCHAEFER, Ann Arbor. J. Am. Med. Assoc., 135, 15: 983. Dec. 13, 1947.

Up to 80% of cholera-vaccinated humans exhibit brucella agglutinins but the brucellergen cutaneous test is negative. A common antigen exists between the two bacteria—the H antigen of *Vibrio comma*. Some 3 million veterans have been vaccinated against cholera. In this study 100

cholera-vaccinated persons were observed. Fifteen were in good health; 85 were patients in a U. S. Navy Hospital for various reasons, but 6 of these were judged to have no disease. Dates of vaccination were known in 74 cases and ranged from 6 to 28 months, with an average of 13 months. Examination by the Huddleson rapid-slide agglutination test revealed 54% positive in a dilution of 1:20 or higher, 41% positive at 1:40 or higher, and 20% positive at 1:80 or higher. This is compared to a value of 2% positive at a dilution of 1:25 or more on 11,000 Wassermann serums. Of these known to have been vaccinated 18 months before the brucella agglutination, 27% were positive at a dilution of 1:40 or higher. Emphasis is given to the effect cholera vaccination may have on the diagnosis of brucellosis in man.

D.P.G.

### MILK

55. Future of H.T.S.T. pasteurization. C. A. WEBER, N. Y. State Dept. of Health, Albany. Milk Dealer, 37, 1: 144. Oct., 1947.

A brief history of H.T.S.T. pasteurization is given and some needed changes are discussed. The following predictions are made: (a) High-temperature short-time pasteurization will be the common method of pasteurization in all but the smaller plants until some entirely new method is developed. (b) The temperature may go higher and the time will be shortened, thereby reducing the size of the holding chamber. (c) Health officials and operators will demand a greater degree of standardization of design and uniformity of requirements but will welcome and accept changes of proved merit. (d) Thermal and safety controls will be more sensitive, responsive and dependable. Instruments will be simplified and combined to control and record automatically both temperature and time of treatment. (e) All surfaces contacted by the product will be streamlined and finished so that less milk solids will accumulate. Mechanical washing will be improved, thereby reducing manual labor and cleaning cost. A practical means or method of starting and finishing an operation without the intermixing of milk and water would be very desirable.

C.J.B.

56. Frozen whole milk. W. A. KRIENKE, Univ. of Ill., Urbana. Milk Dealer, 36, 12: 45, 68-72. Sept., 1947.

The information available at the present time would seem to indicate that, from the standpoint of processing, storage and reconstitution for fluid milk uses, frozen whole milk either in the unconcentrated or in the concentrated form has a promising future. A high quality milk supply is the first essential, as it is in market milk. A high preheating temperature of the milk (170-180° F.) is desirable. Copper contamination should be avoided. If the milk is condensed, a concentration ratio of 3 to 1 is satis-



factory, and the condensed milk should be homogenized at a pressure of 2,000 to 3,000 lb./in.<sup>2</sup> when using the piston type homogenizer. Addition of a small percentage of dextrose has no appreciable effect on the quality of frozen condensed whole milk; its use is not recommended.

The homogenized whole milk or condensed whole milk should be cooled to approximately 40° F. after processing and immediately filled into the proper containers. Effective sealing of the container is essential in order that the entire package has a relatively low moisture vapor transfer rate. Apparently freezing of the products can be done most effectively in a freezing tunnel operated with a blast of air at temperatures of -10 to -15° F. or lower. The frozen products should be transferred from the freezing tunnel directly into the storage chamber. A low storage temperature (-15° F. or lower) is very essential, and a minimum fluctuation of the storage temperature also is of great importance. If transfer of the products to a refrigerated storage having a temperature above -10° F. is necessary, complete defrosting should precede the elevated storage, which should be slightly above freezing.

Defrosting and reconstitution of the frozen condensed milk should be done by placing the frozen block into the proper volume of water at 165-180° F. and allowing it to melt without agitation. For a product of the coffee cream or cereal cream type, proportionately less water should be used than for normal fluid milk. By properly adjusting the concentration ratio of the condensed milk before packaging, it will be possible to use convenient quantities of water for reconstituting a unit of the frozen condensed milk into either of these products. Specific instructions for defrosting and reconstituting should appear on the package. C.J.B.

**57. Control of vitamin D milk.** CURRENT COMMENT. J. Am. Med. Assoc., 134, 17: 1486. Aug. 23, 1947.

In 1933 the Council on Foods and Nutrition of the American Medical Association began to grant acceptance to bottled fresh milk fortified with vitamin D. To secure the Seal of Acceptance of the Council, dairies are required: (a) To declare on label or bottle cap the source and unitage of vitamin D, (b) to have their advertising copy approved by the Council, and (c) to submit proof to the Council in the form of bioassay reports attesting that the milk has been assayed by a reputable laboratory and found to be up to the required potency of 400 units of vitamin D per quart. Many concerns that sell vitamin D milk use the Council's Seal and abide by the regulations, but purveyors of vitamin D concentrates sometimes sell their products without mentioning the need for routine control to assure the physician and the public that proper potency is maintained. Apparently no checkups are made unless required by official agencies or by the Council. The only states having such regulations and providing bio-



logical testing of such milks are Conn., N. Y., Ky., Va. and Wis. Chicago, Cleveland, Detroit, St. Louis and New York are among cities requiring frequent tests. Since vitamin D milks are now depended on by physicians to supply vitamin D, it is suggested that physicians should inquire of their local or state health departments to determine whether such milks are subjected to routine control and whether such regulations exist for the protection of the public and the medical profession. D.P.G.

58. Should we have a single standard of milk quality? G. M. TROUT, Michigan State College, East Lansing. *Milk Dealer*, 36, 12: 134-138. Sept., 1947.

The author concludes that a single minimum standard of quality for milk would be a blessing to the dairy industry and to mankind in general. All the milk solids then could be used for human consumption rather than converting some of them to cheaper animal feeds. Many states now have such a minimum quality standard for milk, but the regulations cannot be enforced at the origin of production due to the inadequacy of personnel. The burden of enforcement cannot be borne by regulatory officials alone. It must necessarily rest in large part with the milk buyer. The legal maxim, "Let the buyer beware", soon may apply to milk buyers as it does to persons in other industries. The time is at hand when all milk solids, either for bottle or for manufacturing purposes, should meet certain minimum quality standards. C.J.B.

59. The demand side of the milk market. L. SPENCER, Cornell Univ. *Milk Dealer*, 37, 1: 116-122. Oct., 1947.

A discussion of the effect of exports, military use, and civilian consumption on the national consumption of dairy products during the past 6 or 7 years is presented. A report then is given on the fluid milk sales in Buffalo, Rochester, and New York City, and of the per capita consumption of milk and cream in the New York-New Jersey Metropolitan area. During the war period the U. S. Government was a heavy buyer of dairy products, taking nearly one-sixth of the total output in some years. Government buying has been reduced drastically and will not be resumed on a large scale except to prevent prices of dairy products from falling to disastrously low levels. Civilian consumption per capita of most dairy products now is much above the prewar level, due largely to high consumer incomes which stimulate the demand for choice foods. Higher retail prices since the end of price ceilings and subsidies last year, combined with increasing supplies of other products, have caused some decline in per capita purchases of fluid milk and cream and ice cream. Butter consumption has increased with the appearance of more adequate stocks but remains far below the prewar level. Per capita consumption of all butterfat is below prewar, while consumption of nonfat solids is nearly one-fourth larger.

Fluid milk sales in the cities of New York State increased about 25 to 50% between 1939 and 1946 but have suffered a slight decline during the last year. Per capita consumption of fluid milk in the New York-New Jersey Metropolitan area reached the highest level in 1945 at 0.93 pint daily. It declined to 0.90 pint in 1946 and will be lower in 1947, although still fully 10% above the prewar rate. Per capita consumption of cream in New York is at least 25% lower than the prewar rate. Even at the higher retail prices now in effect, fluid milk is cheap in relation to the enlarged incomes of industrial workers. A week's earnings of factory workers in New York City now will pay for about one-fifth more milk than could be purchased with prewar earnings. Whether the demand for milk and other dairy products will remain at or near the recent high level depends largely upon the continuance of industrial prosperity and full employment. If business activity and employment should decline, the demand for milk and other choice foods might be affected more seriously than was the case when consumption rates for these products were lower. C.J.B.

60. Campaign of Pennsylvania milk distributor explains where the milk dollar goes. D. S. ADAMS, St. Lawrence Dairy Co., Reading, Pa. Milk Dealer, 37, 1: 42, 43. Oct., 1947.

The method used by the St. Lawrence Dairy Co. to inform their customers, dairy employees, and milk salesmen of the economic factors which operate in the production, processing and delivery of milk is explained. The method is known as the eight-point consumer relations program and is as follows: Ad no. 1 as well as bottle hanger no. 1 points out that the farmer gets 60 cents out of every dollar spent by the consumer for milk. The second ad stresses the fact that dairy employees get 24.5 cents out of each dollar the consumer spends for milk. The third ad reveals that it costs 5 cents to run the dairy, 2 cents for office operation and 3 cents for plant maintenance. The fourth ad explains it cost 3.5 cents for delivery of milk, the fifth that bottles and containers cost 2 cents, the sixth that 2.5 cents is spent for employee pension and insurance funds, and the seventh that 1.5 cents is paid in taxes. The eighth and final ad of the series reveals the usually surprising information that it costs almost 99 cents to deliver a dollar's worth of milk to the consumer's door, and only 1 cent, or one-fifth cent per quart, remains for profit! Each of the ads also emphasizes the value and relatively low cost of milk. "Milk", each ad repeats, "gives you the biggest food value for your dollar." C.J.B.

61. Liquid cooling unit. D. L. KAUFMAN. (Assigned to General Motors Corp.) U. S. Patent 2,431,484, Nov. 25, 1947 (7 claims). Official Gaz. U. S. Pat. Office, 604, 4: 675. 1947.

Liquids in bulk containers, *e.g.*, milk or cream in 10-gallon cans, are



cooled rapidly, conveniently and efficiently by a device consisting of a submerged cylindrical evaporator in which a compressed refrigerant is allowed to expand and evaporate. Provision is made to mechanically raise and lower the evaporator and to agitate the liquid to be cooled by means of a propeller just below the evaporator. When not in use the evaporator is protected by a shield comprised of telescoping sections which automatically extend and contract as the evaporator is lowered and raised.

R.W.

### PHYSIOLOGY

62. The blood groups of cattle. L. C. FERGUSON. J. Am. Vet. Med. Assoc., 111, 849: 466-469. Dec., 1947.

Repeated therapeutic transfusions in cattle often are dangerous; however, most animals can sustain at least one transfusion without clinical response. Reactions usually are characterized by muscular trembling, salivation, lacrimation, coughing, hemoglobinuria, general depression, and temperature elevation to 104-105° F. The abortion of the foetus also resulted in 3 cases. The symptoms, in general, resemble rather closely those in human cases where repeated transfusions of compatible blood are used. The severity of reaction may be decreased in some cattle by the intravenous injection of adrenalin. Genetic studies of cellular antigens have revealed several useful methods of parental identity in cattle. The most positive conclusion at the present time is that a calf may possess a particular antigen in its blood only if one or the other or both of the parents possess that antigen. This method of parentage determination already has been used to good advantage in many cases. The possibility also exists that the genes determining blood groups in cattle are linked closely with those for milk production. Investigations are under way to reveal these associations.

T.M.L.

### SANITATION AND CLEANING

63. How to clean heavily contaminated bottles. C. M. MOORE, Cowles Detergent Co., Cleveland, Ohio. Milk Dealer, 36, 12: 43, 44, 124-132. Sept., 1947.

There are two distinct phases to bottle washing, the bottle washer representing the mechanical phase and the cleaner the chemical phase. The duty of the machine is to take the bottles to the cleaning solution, to necessary rinse or pressure jets or brushes, and then deliver the bottles to the inspection conveyor. It is the duty of the cleaner to remove the visible dirt from the bottle, to destroy bacteria, mold and yeast, and then remove itself from the bottle as quickly and completely as possible. When operated properly, the mechanical phase of bottle washing is a fairly well fixed



and established function. Minor mechanical changes sometimes can be affected, such as increasing the time of contact by slower operation, etc. The important thing is to make sure that the bottle washer is operating properly from a mechanical viewpoint, and then make such changes or adjustments as are necessary in the chemical phase. The latter changes are discussed under extraneous matter, time of contact, temperature and strength of solution. Special emphasis is placed on the use of wetting agents. Directions are given for determining the concentration or the amount of these agents to be used and for determining the quantity required for upkeep.

C.J.B.

## MISCELLANEOUS

64. Waste disposal for country plants. T. F. WISNIEWSKI, Wisconsin State Board of Health. *Milk Dealer*, 36, 12: 50-51. Sept., 1947.

The author states that: (a) Spending money on avoiding waste and on utilizing waste products is more economical than spending it on enlarged waste disposal plants. (b) The most common method of milk waste treatment now in use, providing 80-90% reduction in biochemical oxygen demand, consists of the intermittent application of the waste to a filter composed of crushed stone. The treatment plant consists of a holding tank, trickling filter, and settling tank. The construction and operation of such a plant are described.

C.J.B.

65. Selection and applications of trucks. J. N. BAUMAN, White Motor Co. *Milk Dealer*, 36, 12: 41, 42, 100-112. Sept., 1947.

A tremendous waste in motor transport occurs because, in a large proportion of motor truck installations, consideration is not given to the work that is to be done so that the motor truck can be fitted accurately to the job. A truck is entitled to the same consideration in its applicability for the job as a bottling machine in its applicability. The following 8 steps or procedures, arranged in the order in which they should be considered and which, when complete, will answer all questions of truck application, are set forth and discussed: (a) Determination of the work that the truck is going to be required to do. (b) Determination of the horsepower required to perform this work properly. (c) Selection of the correct model and determination of its wheelbase and load distribution. (d) Selection of the proper tire sizes and types for most efficient operation. (e) Selection of the type of axle best suited for the work that is to be done. (f) Determination of the proper rear axle ratio to bring about the greatest over-all economy of operation. (g) Selection of the type of transmission that will give the most efficient operating condition. (h) Selection of the various optional equipment items that will make possible maximum results.

C.J.B.



## ABSTRACTS OF LITERATURE

### BOOK REVIEW

66. Food regulation and compliance. Vol. II. A. D. HERRICK. 655 pp. \$10.00. Revere Publishing Co., New York 4, N. Y. 1947.

This is the second volume of an interpretation of the Federal Food, Drug, and Cosmetic Act as it applies to food. Special attention is given to the adulteration of foods, including that occurring during processing, packaging and shipping. Considerable space is given to problems of administration of the Act and to a description of the powers of those concerned with its enforcement. Activities connected with the control of importation and exportation of foods are discussed, as are methods of inspection of premises and sampling of products. The book describes the regulation of foods under the Act. It is quite general; yet the many examples given illustrate interpretations of specific conditions.

There is no section devoted, as such, to dairy products. They are mentioned incidentally with other types of foods. Its value to persons interested mainly in dairy products lies in the information given concerning the interpretation of the law and in the description of the methods of enforcement.

The chapter headings illustrate the subject matter discussed. They are as follows: Adulteration in Food Products, Harmful Substances in Foods, Contaminated Foods, Insanitary Premises and Processing, Deleterious Containers, Economic Adulteration, Adulteration in Confectionery, Administrative Regulations, Imports and Exports, Emergency Permit Control, Coal-tar Colors, Inspections and Sampling, Enforcement Means and Methods, Offenses and Violations, Criminal Prosecution, Seizure Proceedings, Injunctive Proceedings. There is an appendix entitled "Federal Food, Drug, and Cosmetic Act and General Regulations for Its Enforcement" in which the drug and cosmetic sections and general regulations are omitted.

M.P.B.

### BACTERIOLOGY

67. The effect of variations in technique on the plate count of milk powders. A. H. WHITE, Div. Bacteriology and Dairy Research, Dept. of Agr., Ottawa, Canada. *Sci. Agr.*, 27, 9: 405-413. Sept., 1947.

A study on the influence of type of diluent, temperature of diluent and temperature of incubation in the bacteriological analysis of milk powder by the plate method is reported. Spray process whole milk powders and spray and roller skim milk powders were analyzed. The use of 0.1N



lithium hydroxide as a diluent greatly reduced plate counts, probably due to the high alkalinity or pH of the solution. Even when diluted 1:100, the pH values of reconstituted milks averaged 9.65. Reconstituting the milk with the diluent at 50° C. resulted in increased plate counts as compared to using a diluent at room temperature. An incubation temperature of 32° C. as compared to 37° C. had little effect on the counts. However, the lower temperature is recommended if available. O.R.I.

68. Sur une méthode simple d'isolement et d'étude des ferments de l'arome des beurres. (On a simple method for isolation and study of aroma-producing ferments of butter.) JEAN KEILLING AND A. CAMUS. Lait, 27, 265-266: 235-237. May-June, 1947.

Based on the premise that aroma-producing organisms develop more satisfactorily in a medium of diluted buttermilk, a method has been devised for the isolation and examination of organisms of this type. One milliliter of serum from butter to be tested is transferred to 1% sterile glucose solution at 20° C. After being shaken at intervals for 2 hr., 1-ml. transfers are made to sterile milk diluted 50, 75, 90, 95 and 98% with sterile water. These tubes are maintained at 18° C. for 24 hr. The contents of each tube then are examined microscopically and diacetyl content determined. Cultures showing desirable properties on these tests then are purified by serial dilution and propagation on solid media. O.R.I.

69. Method of carrying out fermentation processes for production of riboflavin. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,433,063, Dec. 23, 1947 (4 claims). Official Gaz. U. S. Pat. Office, 605, 4: 622. 1947.

The synthesis of riboflavin by the fermentation of sterilized whey or skim milk by *Clostridium acetobutylicum* is improved by having the iron content in excess of 0.1 and 0.21 p.p.m. and the pH maintained in a definite range depending on the quantity of iron. R.W.

70. Method of preparing riboflavin from whey and skimmilk. N. E. RODGERS, H. L. POLLARD, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,433,064, Dec. 23, 1947 (4 claims). Official Gaz. U. S. Pat. Office, 605, 4: 623. 1947.

Essentially the same as U. S. Patent 2,433,063 (see preceding abstract) except that the pH of the medium is adjusted to 5.8 to 6.5 with lactic acid prior to sterilization. R.W.

71. Method of preparing riboflavin from lacteal material. R. E. MEADE, N. E. RODGERS, AND H. L. POLLARD. (Assigned to Western Condensing Co.) U. S. Patent 2,433,232, Dec. 23, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 605, 4: 667. 1947.

The production of riboflavin from whey or skim milk by *Clostridium acetobutylicum* is increased by incorporating xylose in the medium.

R.W.

### BREEDING

72. The causes and diagnosis of infertility in bulls. G. R. MOORE. J. Am. Vet. Med. Assoc., 112, 850: 25-29. Jan., 1948.

The causes of sterility in the bull may fall logically into 3 groups: (a) those caused by inflammatory processes of the testes and of the tubular genitalia, (b) testicular hypoplasia and degeneration due to hormone deficiencies and other causes, and (c) functional disturbances due to systemic ill health, senility, malnutrition, obesity and lack of exercise. Certain precautions in management and breeding may greatly improve the fertility of sires. Selecting for reproductive efficiency and good fertility is extremely important in any breeding program. It also is a good management policy to provide the young sire with adequate green feeds, protein supplements, minerals, and an opportunity for an abundance of exercise for best breeding efficiency.

T.M.L.

73. Cost of getting cow with calf. W. F. SCHAEFER. Guernsey Breeders J., 73, 1: 11-13. Jan. 1, 1948.

The cost of keeping bulls at the Nepa Artificial Breeding Cooperative, Tunkhannock, Pa., averaged \$614 in 1946. This amount included: Lease fee and depreciation of bulls owned (depreciation 50% per year) \$222, feed \$175, labor \$150, housing \$37, bedding \$12, trucking \$11, veterinary \$7. During the year, 23,141 cows were bred at a cost of \$3.95 per cow offered for service. An average of 1.53 inseminations was required per cow.

A.R.P.

### BUTTER

74. A fluorescence method for assessing the keeping quality of butter. G. A. GRANT AND W. HAROLD WHITE, Natl. Research Laboratories, Ottawa, Canada. Can. J. Research, F, 24: 461-466. Nov., 1946.

A method was developed for determining the fluorescence of the diluted sera of butter samples. Fluorescence values were found to be related to flavor scores of salted butters which had been stored at high temperatures. The recommended procedure was as follows: The serum was separated by placing 125 g. of butter in centrifuge bottles and heating in a boiling water bath, centrifuging at 1700 r.p.m. and siphoning off the fat. Two ml. of the



serum was diluted to 50 ml. with 10% sodium acetate, the pH adjusted to 5-6, and the fluorescence values determined immediately in a Coleman photofluorometer using a filter that transmitted light in the region of 365 m $\mu$ . This procedure gave fluorescence values that were correlated with flavor scores ( $r = -0.84$ ) on salted butters stored at 32.2° C. (90° F.) and sampled at intervals during 32 days. O.R.I.

75. Contribution a la connaissance du beurre de brebis (beurre de cashcaval) prepare en Roumanie. (Contribution to the knowledge of sheeps' butter (cashcaval butter) prepared in Roumania.) C. STOIAN. Lait, 27, 267: 342-352. July-Aug., 1947.

In the making of cashcaval cheese from sheeps' milk, the curds are heated by hot water and high losses of fat occur. This may be salvaged either by gravity or centrifugal separation. Butter oil prepared from this fat is marketed in southeastern Roumania. Eight commercial samples were analyzed for such physical and chemical constants as melting and solidification point, index of refraction, specific gravity, saponification, Reichert-Meissl, Polenske and other values. Widely varying values were obtained in melting and solidification points, but in other respects fairly uniform values were found. The average R-M value was 29.88 and the average P value 4.53. O.R.I.

## CHEESE

76. Factors influencing the quality of cheese. H. L. WILSON. Can. Dairy Ice Cream J., 26, 11: 45. Nov., 1947.

Sanitation is the most important factor confronting the cheese industry. The operator of a clean, sanitary plant should not accept milk of inferior quality. All bacteria foreign to cheesemaking should be eliminated. The control and proper rate of acid development are important in the making of a good uniform quality of cheese. The time element also is very important; 2.25 hr. should elapse from setting to drawing the whey, and at least 2.25 hr. from time of drawing whey to milling. To make a cheese that is uniform in quality as well as type, a uniform method and time schedule must be used, varying only those steps that are necessary because of bacterial action. H.P.

77. Rheological experiments carried out on Gruyere. G. MOCQUOT, G. W. S. BLAIR, AND M. BARON. Natl. Inst. for Research in Dairying, Shinfield, England. Dairy Inds., 12, 10: 966-976. Oct., 1947.

The measurement of superficial density used for Cheddar cheese manufacture in England has been applied to the production of Gruyere type cheese made in French Jura. The method (described in a previous publication in 1940), which measures the superficial density of the curd, was found



to be very valuable to cheesemakers, particularly when they are confronted with the processing of abnormal milks (mastitis, high acid, etc.).

Measurements for plasticity and elasticity by means of a ball compressor during curing constitute a valuable tool in predicting the future quality of cheese. With this information the cheesemaker can then correct or prevent certain defects from developing by such procedures as accelerating or retarding ripening.

D.V.J.

78. Apparatus for use in the centrifugal separation of serum from cheese constituents. G. J. STREZYNSKI. (Assigned to De Laval Separator Co.). U. S. Patent 2,432,829, Dec. 16, 1947 (13 claims). Official Gaz. U. S. Pat. Office, 605, 3: 507. 1947.

A centrifuge has been developed which continuously removes whey from a cultured milk-cream mixture to form cream cheese. The mixture passes through a revolving bowl which is provided with 2 outlets, one for the lower density constituent whey and the other for the concentrated curd and fat. The movement of the latter is facilitated by a scaper or conveyor and by maintaining the incoming temperature of about 160° F. through suitable insulation of the equipment. The mechanism is so designed that the curd and fat mixture is not aerated as it continuously leaves the bowl and moves through the supplementary devices. Measured amounts of such desirable additives as gum, salt and flavoring ingredients may be continuously injected into and intimately mixed with the cream cheese as it is discharged from the machine.

R.W.

#### CHEMISTRY

79. Différenciation de la caséine et de la lactalbumine par un processus microbien. (Differentiation of casein and lactalbumin by a microbial process.) JEAN KELLING AND A. BARRET. Lait, 27, 267: 337-342. July-Aug., 1947.

In the course of studying dairy fermentations, a mycoderm was isolated which possessed the ability to digest casein to amino acids. No amino nitrogen, as determined by the Sorensen method, was produced in media in which albumin was the source of nitrogen. The organism on solid media produced small round colonies with irregular contours. The cells were elongated, 3-5  $\mu$  in diameter and 5-6  $\mu$  in length. They reproduced by budding. When cultured in milk, a maximum yield of amino nitrogen was obtained in 16 days at 30° C. The total lactose of milk was not greatly reduced after 16 days. It is suggested that this organism will be of value in laboratory determinations and in the ripening of soft cheese. O.R.I.

80. Analyse des crèmes. (Analysis of creams.) R. MOREAU. Lait, 27, 265-266: 257-258. May-June, 1947.

A modification of the Koehler-Bacot method is proposed whereby the

sulfuric acid and water would not be added separately to the butyrometer but would be mixed in the proportion of 5 ml. of water to 10 ml. acid prior to being added. After adding amyl alcohol, the tubes are placed in a water bath at 85° C. to facilitate digestion. The fat columns are measured at 65° C. O.R.I.

### DISEASES

81. Mastitis. R. F. WAECHTER. *Can. Dairy Ice Cream J.*, 26, 11: 90. Nov., 1947.

Chronic mastitis, the type most frequently found, is defined as a progressive inflammation of the udder or mammary gland. From this infection, the milk secreting tissues in the gland gradually are destroyed. Mastitis can be detected by the strip cup, manipulation of the udder, or by bacteriological tests. Methods of control require hygienic stable and milking conditions. Newer methods of treatment include the use of the newer sulfa drugs and penicillin. The use of mastitis mixed bacterins in the treatment and prevention of mastitis is recommended. The advice of a veterinarian should be obtained when the disease is observed. H.P.

82. Streptomycin in the treatment of calf pneumonia. R. F. VIGUE. *J. Am. Vet. Med. Assoc.*, 111, 848: 389-390. Nov., 1947.

In 7 cases of calf pneumonia, 3 of which were complicated with diarrhea, all terminated favorably after streptomycin was used in addition to blood transfusion and oral sulfadiazine therapy. T.M.L.

83. Treatment of pneumonia in cattle. S. J. ROBERTS AND G. K. KIESEL. *J. Am. Vet. Med. Assoc.*, 112, 850: 34-39. Jan., 1948.

Treatment with sulfamerazine, sulfamethazine and penicillin resulted in the recovery of 94.6% of 129 cases of the pneumonia form of hemorrhagic septicemia in older cattle; 93 calves treated in the same fashion gave 81.7% recovery. Although no animals were kept untreated for checks, evidence indicates that treatment with the newer pyrimidines has been somewhat effective in reducing losses of cattle from pneumonia. When sulfamerazine and sulfamethazine were used in the recommended dosages of 0.5 to 0.75 gr. per lb. of body weight daily, no toxic reactions were observed. T.M.L.

84. A further report on staphylococcic abortion in a dairy herd. W. D. POUNDEN, L. C. FERGUSON, C. E. KNOOP, AND W. E. KRAUSS. *J. Am. Vet. Med. Assoc.*, 111, 848: 376-378. Nov., 1947.

In a herd of 50 cows bred artificially to 7 different bulls, 6 out of 15 cows bred to one particular sire aborted. The time of abortion varied from 137 to 242 days. All animals that aborted were inseminated anterior to the cervix. (This procedure was used as a precaution against further in-



fection.) An organism resembling *Staphylococcus albus* was recovered from the necrosed cotyledons or pus in 4 instances. In 2 cases the organism was found in the aborted calves. Organisms of apparently similar characteristics were recovered from semen samples from the bull in question. All cows bred were negative to tests for brucellosis and trichomoniasis.

T.M.L.

### ICE CREAM

85. **Overrun control in ice cream.** P. H. TRACY, Dept. of Dairy Husb., Univ. of Ill. Ice Cream Field, 50, 4: 88, 89. Oct., 1947.

The importance of overrun control is stressed and data given to show the relation of overrun to ice cream ingredient cost. However, ingredient costs are not the only ones affected by producing ice cream with lower overrun. The author states: "The aim of the industry at the present time is an ice cream with an overrun of about 60 percent." Dipped ice cream should have slightly more than 50% overrun; fewer problems result in producing packaged ice cream at an overrun of 60-65% than at 50%. Dipping materially changes the texture of ice cream. Low overrun (40-60%), machine filled packages have a better texture than hand dipped packages with the same overrun. The author suggests that ice cream be frozen to the usual 100% overrun and, after partially freezing, part of the air be pressed out so as to obtain the desired overrun. The economics of the problem requires that the industry ascertain the optimum overrun consistent with quality and the willingness or ability of the consumer to pay.

W.C.C.

86. **The shrinkage problem.** C. D. DAHLE AND J. A. MEISER, JR., Dept. of Dairy Husb., Pennsylvania State College. Ice Cream Rev., 31, 5: 64, 67. Dec., 1947.

In order to obtain information as to the prevalence of shrinkage in ice cream and to learn of methods used by the industry to combat it, a questionnaire was submitted to members of the I.A.I.C.M. The answers indicated that more shrinkage occurred in plants using sweetened condensed milk than when other forms of concentrated milks were used. The elimination of wheat sirups from the mix eliminated shrinkage in 11 out of 18 plants using this product. Eliminating or reducing the amount of other sirups also eliminated a considerable amount of shrinkage. Lowering hardening room temperatures from +5 or -10° F. to -15 or -20° F. was effective in reducing shrinkage in several cases. Freezing ice cream to a lower temperature at the freezer and slowing down the continuous freezer were found helpful in controlling shrinkage. Some plants reported they stopped shrinkage by changing stabilizers, and 3 plants stopped their trouble by eliminating the use of emulsifiers. There is no single remedy for the shrinkage problem.



From results of research work, as well as from information obtained from the questionnaire, it would appear that the use of sweetened condensed milk, the use of certain sirups in the mix, the presence of free fatty acids, the use of emulsifying agents, improper surface of paper containers, improper temperatures in the hardening room and cabinets, and season of year may contribute to shrinkage.

W.J.C.

87. **Vanilla, the edible orchid.** N. C. LARSEN, Polak & Schwarz, Inv., Ice Cream Trade J., 43, 8: 72, 73, 85-88. Aug., 1947.

This article deals with the early history relating to the discovery of the vanilla bean, definitions of the various types of beans, how they are propagated, cured and packaged, quantity consumed, composition and uses.

W.H.M.

88. **Defrosting.** S. RUPPRIGHT. Ice Cream Trade J., 43, 8: 78, 98. Aug., 1947.

Defrosting of refrigeration coils may be accomplished with outside air or with hot gas from the high side of the refrigeration system or brine in a brine system through valved connections. Another method is by use of heat of dissolution liberated by thinning a brine with the substance of the frost. Electrical resistance heat may be used when the resistance wire is thermally united with the coil. Water also is used as a carrier of heat for defrosting evaporator coils. Water defrosting may result in freeze-ups unless done quickly with special equipment. The washing effect of water is desirable and aids in removal of odors from the coils.

W.H.M.

89. **Improving packages.** J. H. ERB, The Borden Co. Ice Cream Trade J., 43, 10: 104, 144, 145. Oct., 1947.

The most common faults of much packaged ice cream are high overrun, weak body, coarse texture, fluffy body and poor flavor. Packaged ice cream should have a fine flavor, smooth texture and firm body. A total solids content of 38.5 to 40% is desirable. The use of corn sirup to gain in non-sweet solids and of high grade egg yolk to produce small air cells is advantageous. Only high quality raw material should be used. Thorough homogenization and 5 hr. of aging are recommended. In freezing, the overrun should be kept under that of corresponding flavors of bulk ice cream. Every container should be of the same weight. The ice cream should be frozen to a stiff dry consistency and transferred to the hardening room quickly. The hardening time should be 6 to 8 hr. Appealing flavor and flavor combinations assist in promoting the sale of packaged ice cream. In addition to standard flavors, 2-layer, 3-layer and revel type of packages are desirable. The type of container from which ice cream must be dipped yields a product more like hand-dipped bulk ice cream, but this style of package lacks the ready serving convenience of the container which is

easily pulled away from the ice cream. Another important factor in the production of satisfactory packaged ice cream is the establishment of an adequate system of quality control.

W.H.M.

90. Automatic ice cream packaging is here. ANONYMOUS. Ice Cream Trade J., 43, 10: 98, 99, 164, 165. Oct., 1947.

Machines for packaging ice cream now are being manufactured by Anderson Bros. Mfg. Co., Rockford, Ill.; Pure-Pak Division, Ex-Cell-O Corp., Detroit, Mich.; Ray Industries, Los Angeles, Calif.; Frank D. Palmer, Inc., Chicago, Ill.; and Prestige Products Co., New York City.

Some of these machines already are being used commercially. Others have been fully tested and are ready for use in the plant. Still others are thoroughly tried refinements of earlier machines. All are designed to set up, fill, and seal various types of containers commonly used in the ice cream industry. All should play their part in ultimately permitting a more rapid rate of packaging with lower cost per unit and more sanitary handling.

W.H.M.

91. A complete report of Breyer's experiences to date with the bulk gallon. A. L. HACKMAN, Breyer Ice Cream Co., Long Island City, N. Y. Ice Cream Trade J., 43, 10: 94, 140, 141. Oct., 1947.

This company first introduced the 1-gallon container for bulk ice cream in the Harrisburg area on Nov. 1, 1945. The first month's sales represented 3.7% of the total sales, the second month 8%, and the third month 12%, which later dropped to 4-5% of total sales. Most dealers were in rural areas. Later the gallon container was introduced in Allentown and Scranton, Pa., Wildwood, N. J., and Salisbury, Md., with sales ranging from 2 to 5.5% of total sales. Experience in the New York area was not as satisfactory, with sales averaging 2%, compared to 4 to 10% in the Philadelphia area. Apparently there is a large consumer demand for bulk ice cream in gallon containers at a reasonable price. The retail price must appear on the container. Dealers are allowed a 20% mark-up.

W.H.M.

92. Ice cream as a nutritious food. J. W. LAWRENCE. Can. Dairy Ice Cream J., 26, 11: 37. Nov., 1947.

Ice cream contains almost 80% dairy products, in the form of milk, cream, and milk solids. An average serving of ice cream compares favorably in mineral and protein content with apple pie, rice pudding, chocolate layer cake, lemon meringue pie, and fruit cup, and is superior to these foods in many categories. An average serving of ice cream contains only 140 calories as against 300 calories in a normal serving of apple pie, 400 in a piece of chocolate layer cake and 450 in a slice of lemon meringue pie. Ice cream is placed in the category of protective foods, containing more



proteins, calcium, vitamin A and riboflavin than many desserts. The protein and mineral content of ice cream is more easily digested than that of other sources. At retail prices it compares favorably with many other staple foods. H.P.

93. Adding smaller markets. ANONYMOUS. *Ice Cream Trade J.*, 43, 10: 96, 138. Oct., 1947.

Since introducing Holly Carter brand of ice cream in Milwaukee and the Crestmont brand in Detroit, the A & P stores are selling ice cream in many smaller communities. In these small markets the stores are selling ice cream purchased from local manufacturers. The usual mark-up is about 22%. No figures are available at this time on sales volume. W.H.M.

94. Drive-in operations. H. J. MULDOON. *Ice Cream Field*, 50, 5: 24-26. Nov., 1947.

The ideal spot for a drive-in is on the outskirts of the business district on a well-traveled thoroughfare used by local people on their way to and from the business district. For a drive-in with seating capacity of 32, a building 20' x 45' on a lot 100' x 165' with parking facilities for 40 cars is recommended.

Ice cream and products containing ice cream should be the main products sold. However, hamburgers and coffee also are essential to the best operation. Care in selecting help is stressed; young married women or divorcees with one or more children are recommended for car hops instead of boys or bobby sockers. Proper training and supervision of employees are emphasized, supervision constituting the most important phase of the whole operation. Labor and supervision costs amount to 20-22%, which is higher than for most inside stores. However, to do the same volume of business with an inside store would require about double the investment required for a drive-in. Customer advantages and complaints and operator advantages and complaints are listed. W.C.C.

95. Planning the operation of retail drive-in ice cream stores. O. H. GLAZIER. *Ice Cream Trade J.*, 43, 11: 66, 67, 92, 93, 94. Nov., 1947; also *Ice Cream Rev.*, 31, 5: 74, 76, 78, 80, 82. Dec., 1947.

The location should provide ample space for the parking of cars and a pleasant background. The stand should be located on the edge of the residential area rather than on a main traffic artery. There may be exceptions to this rule, such as a location that has an unusually attractive background or something of great interest near the stand that people will want to stop and see.

The building should be designed correctly, in proper proportion and in harmony with its surroundings. Some landscaping in the form of shrubs



or flower gardens helps to create the desired impression. Two dipping cabinets arranged at right angles to the front windows are desirable. One should be set at 10° F. for lower melting ice cream like chocolate and fruit ice cream and the other at 14° F. for the higher melting plain ice creams. Paper cups and dishes are best adapted to his type of business because they eliminate washing and breakage. Ample storage space should be provided. A direct expansion ammonia batch freezer is recommended, or a continuous freezer might be considered if some wholesale business is done. Storage space for cones, dishes and fountain supplies should be provided in the stand. No stand is complete without a fountain, and it should be of the proper size for the building. Mixers for milk shakes also are needed. Good wooden spoons and individually wrapped straws help to create good customer impressions.

Personnel must be chosen with care by a process of elimination. The author has had best experience with young men 18 to 25 years of age. They are faster, understand how to do things without being told all the smaller details, and do not get confused as easily in making change and in filling complicated orders during a rush.

Limit sales to ice cream and soda bar products such as sodas, sundaes and milk drinks. High-quality products sold in a friendly manner in pleasant surroundings for a fair price are the best insurance for the success of any retail ice cream store.

W.H.M.

**96. Costs, prices, profits.** A frank analysis of the industry's position today. V. F. HOVEY, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 43, 10: 84, 85, 145-147. Oct., 1947.

The cost of making ice cream in Sept., 1947, increased 11.5¢ per gallon over the Sept., 1946, costs, with no allowance for difference in volume. This 11.5¢ is made up of 4¢ for material, 4¢ for labor, 0.5¢ for depreciation and 3¢ for other items.

Some manufacturers may try to cut costs by increasing overrun. However, the author believes this is a mistake. A reduction in volume of sales will have a marked effect on cost. Figures indicate that a decrease of 10% in volume will result in an increase in cost of about 3¢ per gallon; a 20% decrease in volume increases the cost about 6¢ per gallon.

Since the consumer price is so closely related to consumption, manufacturers may have to print retail prices on their packages unless retailers voluntarily keep percentages of profit at a reasonable level. A sound price structure with a discount scale providing a difference in price to large and small customers, at least on bulk ice cream, equal to the difference in costs of serving them, is advocated. Manufacturers should have printed price lists to which they adhere so that each manufacturer knows the basis upon which competitors solicit business. The discounts to be given to any cus-

tomater should be determined only at the close of the year when his actual gallonage is known. W.H.M.

97. Frozen dessert composition. S. MUSHER. U. S. Patent 2,431,704, Dec. 2, 1947 (7 claims). Official Gaz. U. S. Pat. Office, 605, 1: 66. 1947.

As a means of controlling the overrun, avoiding bleeding and improving the texture and body of sherbets, ices and ice cream, a stabilizer consisting of oat flour is recommended. To obtain optimum results, a premix should be prepared by blending the oat flour, gelatin if used, part of the sugar and some of the water or milk of the mix and heating to at least 175° F. and preferably to 190-220° F. This mixture then is combined with the other ingredients of the mix prior to pasteurization. About 1% of the total mix should be oat flour. R.W.

98. Ice cream and ice cream mix. T. B. HIPPLE AND S. S. SADTLER. U. S. Patent 2,433,276, Dec. 23, 1947 (8 claims). Official Gaz. U. S. Pat. Office, 605, 4: 677. 1947.

A dry powder, suitable for making ice cream mix, is prepared by spray drying a mixture of hydrogenated butterfat, cream and soya protein. R.W.

### MILK

99. The freezing point of milk: Some recent developments F. J. MACDONALD. Dairy Inds., 12, 9: 846-851. Sept., 1947.

The developments that have taken place since the early work of Dreser in 1892 on the freezing point of milk and its application are discussed. The average freezing point of 480 samples of milk taken from tank car shipments over a period of 2 years was -0.544° C. This figure agrees with those previously reported by most investigators.

Mastitis milk has essentially the same freezing point as normal milk, while the development of acidity increases the depression of the freezing point. The use of formalin also increases the depression of the freezing point but can be corrected for by calculation. Data from previously published work show the amount of correction required when various amounts of formalin are added. D.V.J.

100. L'irradiation du lait aux Etats-Unis. (The irradiation of milk in the United States.) C. WOLF. Lait, 27, 265-266: 238-257. May-June, 1947.

Irradiation methods and literature in the United States up to June, 1939, are reviewed. In a supplementary note, standards and practices adopted since that date are described. O.R.I.



## PHYSIOLOGY

101. Why hormone treatments sometimes fail. C. F. CAIRY. J. Am. Vet. Med. Assoc., 112, 850: 30-33. Jan., 1948.

The infancy and complexity of the hormone field make it a dangerous one for inexperienced workers. The lack of fundamental physiologic knowledge is one of the serious handicaps of hormone therapy. The exact nature of hormones and their specific action in the animal body still are unknown. Certain precautions are fundamental in any hormone therapy. Make as complete a diagnosis as possible. Evaluate environmental and clinical factors which may have same symptoms as hormone dysfunction. Use only products whose strength has been determined. Dosage is most important, since excesses often produce opposite effects. Keep up on species differences.

T.M.L.

## SANITATION AND CLEANSING

102. Some aspects of detergency involving surface chemistry and physics. J. C. L. RESURGGAN, The British Hydrological Corp. Dairy Inds., 12, 9: 852-855. Sept., 1947.

The fundamental chemical and physical aspects of detergency are discussed. The process cannot be defined in terms of either physics or chemistry alone, since many of the physical effects are dependent upon chemical reactions in the detergent solution or at the interface between the solution and the deposit. The subject is discussed in the light of established physical laws and chemical reactions and the parts they play in the complex forces involved in the removal of deposits from metals, glass and plastics.

D.V.J.

103. Cleansing of dairy utensils. III. Results of the bacteriological examination of rinses and swabs of farm dairy utensils. S. B. THOMAS, P. M. HOBSON, C. G. JONES, E. JONES-EVANS, AND J. C. DAVIES. Dairy Inds., 12, 11: 1095-1099. Nov., 1947.

This investigation was undertaken to study the "rinse" and "swab" techniques of checking washed utensils on English dairy farms. Two types of farms were studied, namely, control farms using routine steam sterilization of utensils and Category C farms (600 in number) which frequently produced reject class milk.

Over 87% of rinses on control farms showed less than 50,000 bacteria, while only 31% of the Category C farms fell in this class. With swab tests, 88% of the control farms had colony counts under 5,000 per sq. ft. as compared with 27% for the Category C group. Results showed that 50% of utensils on Category C farms were grossly contaminated. The authors conclude that the swab technique is in many ways superior to the rinse method.

D.V.J.



104. Germicidal effect of quaternary ammonium compounds on dairy organisms. R. V. HUSSONG. Can. Dairy Ice Cream J., 26, 11: 92. Nov., 1947.

The advantageous properties the quaternary ammonium compounds possess for germicidal purposes are that they are soluble in water, non-corrosive, non-toxic, chemically stable, have rapid killing action, are non-volatile, are practically odorless and tasteless, are wetting agents, and have high germicidal action on test organisms used. One disadvantage of these compounds is that they are not compatible with certain alkalis and soaps used in the dairy plants. If surfaces are not rinsed free of the non-compatible materials, they may neutralize the effect of the quaternary compounds. Experimental results indicate that Roccal, Ster-Bac, Emulsept, and Isothan all were effective against *Streptococcus lactis* and *Staphylococcus aureus* and against two species of yeasts, *Torula cremoris* and *Torula sphaerica*. H.P.

105. Control of insects and rodents in food plants. G. C. DECKER. Can. Dairy Ice Cream J., 26, 11: 78. Nov., 1947.  
See Abs. 134. J. Dairy Sci., 30, 4: A61. April, 1947.

#### MISCELLANEOUS

106. What the Federal Food and Drug is looking for. C. T. HUBBLE, Minneapolis Station, Food and Drug Administration. Natl. Butter Cheese J., 39, 1: 42, 44, 46. Jan., 1948.

The Federal Food, Drug and Cosmetic Act of 1938 differs significantly from the Act of 1906 in requiring that foods be manufactured under sanitary conditions. The terms of the Act are general and are not restricted to conditions affecting the health of the consumer. Inspectors of food plants are instructed to point out failures in sanitary control to interested manufacturers. The sediment test for insoluble material and the methylene blue test for bacterial load are practical tests applied to examine the condition of milk. Penalties for violation may include seizure of the product, criminal prosecution of those responsible for violations, or injunction to cease violating the law. Fines and prison sentences may be imposed.

W.V.P.

107. One dairy plant operator influences 844 farmers. ANONYMOUS. Natl. Butter Cheese J., 39, 1: 30, 31, 64. Jan., 1948.

A survey of representative members of the dairy industry in 27 different states disclosed that 98% of them sell supplies and equipment to farmer patrons. Such sales are made to provide desirable facilities and to give fieldmen the chance to demonstrate proper methods of production. Over half of the sales are made at cost; 42% are made at regular retail prices.

Washing powders, filter discs, milk strainers, milk cans, brushes, insect sprays and sterilizers are the items most commonly handled. W.V.P.

108. Practical training for dairy technologists. C. K. JOHNS. Can. Dairy Ice Cream J., 26, 12: 21. Dec., 1947.

Theoretical knowledge is necessary but it must be supplemented by a good background of practical experience. The average college graduate was found to lack practical experience. The dairy industry must recognize that the provision of practical training is primarily its responsibility. If the dairy industry is to obtain the right type of man for positions of responsibility, some thought should be given to a program of student training. The need for well-trained men will increase from year to year. H.P.

109. West coast dairy cooperative tests key men on supervision. ANONYMOUS. Food in Canada, 7, 7: 15-18. July, 1947.

During the winter months the Okanogan Valley Cooperative Creamery Association conducts classes designed to impress foremen and supervisors as to the importance of good public relations and the necessity of careful supervision of employees. A series of 50 questions dealing with practical problems in these fields are presented in the article. Many of the questions present a number of alternative situations designed to allow the employee to appraise himself for positions of management. O.R.I.

110. New type refrigerator car requires no ice. E. M. HOLLER. Food in Canada, 7, 7: 20-22. July, 1947.

A new type of railroad refrigerator car employing an absorption ammonia system for cooling is illustrated and described. Tanks holding 1,900 lb. of liquid anhydrous ammonia are slung beneath the car. The flow of ammonia to the expansion coils in the car ceiling is controlled by bulb-type temperature control apparatus. Later the ammonia is absorbed in water in a tank slung below the car. In the test reported, the car maintained temperatures at approximately 0° F. for a 10-day trial in which the car was held in a test house at 90° F. The car was fully loaded with frozen foods and these remained in a satisfactory condition. Costs for operating this type of equipment have not been determined. O.R.I.





## ABSTRACTS OF LITERATURE

### BOOK REVIEWS

111. Annual review of microbiology. Vol. I. C. E. CLIFTON, editor. 404 pp. \$6.00. Annual Reviews, Inc., Stanford, Calif. 1947.

This volume initiates another series in the tradition of the "Annual Review of Biochemistry" and the "Annual Review of Physiology". Each chapter is written by one or more specialists in that particular field. The chapter on Bacterial Metabolism is reproduced from the current volume of "Annual Review of Biochemistry". Although much of the material covered is primarily of interest to specialized medical bacteriologists, the chapters on Bacterial Metabolism, Nitrogen Metabolism, Industrial Fermentations and Quaternary Ammonium Compounds contain considerable material of interest to those in the dairy industry, particularly dairy bacteriologists. Those concerned with animal health will be interested in the chapters on Antibiotics, Chemotherapeutic Agents, Immunochemistry, Some Aspects of Active Immunization and probably several others. The indexing of the book seems very satisfactory, and the numerous literature references permit the reader to consult the original publications with a minimum of difficulty.

F.E.N.

112. Advances in enzymology. Vol. VII. F. F. NORD, editor. 665 pp. \$8.75. Interscience Publishers, Inc., New York, N. Y. 1947.

The broad viewpoint of this volume is indicated by the fact that 5 of the 12 chapters are written by men from European countries. The approach definitely is fundamental, as each chapter covers in considerable detail the status of present knowledge in a comparatively restricted field. The chapters are titled: Permeability and Enzyme Reactions; The Properties of Protoplasm with Special Reference to the Influence of Enzymic Reactions; Recent Views on Asymmetric Synthesis and Related Processes; Some Applications of Radioactive Indicators in Turnover Studies; Bacterial Luminescence; Heme-linked Groups and Mode of Action of Some Hemoproteins; Distribution, Structure, and Properties of the Tetrapyrroles; Oxidation of Organic Sulfur in Animals; Interrelations in Microorganisms between Growth and the Metabolism of Vitamin-like Substances; Antibacterial Substances from Fungi and Green Plants; Kidney Enzymes and Essential Hypertension; and Recent Progress in Industrial Fermentation. In addition to the literature citations for each chapter, author and subject indices for this volume and cumulative author and subject indices (only for chapter titles and chapter authors) for the 7 volumes published to date are included.

F.E.N.

## BACTERIOLOGY

113. Microbiological assay for riboflavin. H. A. KORNBERG, R. S. LANGDON, AND V. H. CHELDELIN, Dept. of Chem., Oregon State Coll., Corvallis. Anal. Chem., 20, 1: 81-83. Jan., 1948.

Riboflavin is determined using *Leuconostoc mesenteroides* 10,000. The response of the organism to riboflavin permitted the development of an assay method which is sensitive to 0.0001  $\gamma$  of the vitamin per ml. Good agreement is obtained among riboflavin values of samples assayed at different levels as well as good recoveries of added riboflavin. Determinations may be made turbidimetrically after 14 hr. or titrimetrically after 72 hr.

B.H.W.

## BUTTER

114. Influence of separator cleanliness-storage temperature on cream quality. J. M. JENSEN AND A. L. BORTREE, Mich. State Coll. Am. Butter Rev., 9, 5: 10, 12, 14, 16. 1947.

Cream from clean bowls held at 92 score for 72 hr. when stored at 53° F., while that from dirty bowls held a 92 score only 48 hr. When stored at 63° F., cream from the clean bowl had an average flavor score of 90.3 after 72 hr., while that from dirty bowls was maintained at 90 score for only 48 hr. Cream held at 73° F. and separated from the dirty bowl stored at 73° F. maintained a 90 flavor score for only 24 hr. Essentially as much acid was developed in 24 hr. with cream stored at 73° F. as was developed in cream stored at 53° F. for 96 hr.

Frequent delivery of cream, clean separators, and proper cooling are essential to quality cream production. Of the 3 conditions, proper cooling is by far the most important; temperatures of 53° F. or less are advisable. When cream was stored in a household refrigerator at 40° F., control of acidity development and flavor deterioration was satisfactory up to 96 hr.

P.S.L.

115. Sediment testing of cream. A. W. RUDNICK, Iowa State College, Ames. Am. Butter Rev., 9, 12: 22, 24. 1947.

Samples for sediment testing other than those taken off the bottom of unstirred cans of cream are unsatisfactory. The following procedure has been developed to facilitate proper sediment testing of cream. Using an off-bottom tester without pad, the sample is taken from the bottom before the can is stirred. The sample then is discharged into a suitable vessel and mixed with 12 to 16 oz. of filtered water. A pad is placed in the tester and as much of the mixture drawn up as possible and discharged. The operation then is repeated with the remainder of the sample without



changing the pad. In some cases it may be necessary to dilute the sample with additional water. P.S.L.

116. The dual use of the vacreator. G. H. WILSTER. Oregon State Coll. Am. Butter Rev., 5, 13:28, 30, 32. 1946.

A comparison was made at Oregon State College of butter from 86 churnings of cream from each of 2 pasteurization methods. Butter from cream pasteurized in the vacreator had an average score 0.83 point higher than butter from cream pasteurized by the vat method. Destruction of bacteria was more complete with the vacreator system of pasteurization. Similar experiments were carried on by the University of Manitoba and Iowa State College. At the University of Manitoba, in 153 comparisons, all but 8 churnings resulted in a higher flavor score when the vacreator system of pasteurization was used. Experimental results at Iowa State showed butter made from vacreator-pasteurized cream to have an average score 0.97 point higher than butter made from vat-pasteurized cream.

A study also was made at Oregon State as to the possibilities of pasteurizing ice cream mix with the vacreator. Ice cream of highly satisfactory flavor, body, texture, and melt-down characteristics was made from mix prepared by condensing the required skim milk to the proper density through the use of the vacreator, adding the other mix ingredients to the condensed skim milk, and subjecting the mixture to homogenization, vacuum pasteurization with the vacreator and cooling. The thermal efficiency of the Oregon State unit was 50.3%, but it is estimated that it could have been 85% had an efficient heat exchanger been used. The cost of removing water from skim milk was calculated to be 0.193 cent per lb.

P.S.L.

117. Cream separator. C. E. DEARDORFF. (Assigned to C. E. Deardorff, Inc.) U. S. Patent 2,434,642, Jan. 20, 1948. Official Gaz. U. S. Pat. Office, 606, 3: 439. 1948.

A container for milk is described. It has a horizontal partition so positioned that the upper compartment thus formed is of such volume that it contains substantially all of the cream. A flap is attached to the under side of the partition which closes a hole in said partition when the container is tipped in a designated direction. The skim milk is retained in the lower compartment by the flap sealing the opening, while the cream is poured out of the upper compartment through a hole in the top. R.W.

## CHEESE

118. Manufacture of cottage cheese. A. A. SCHOCK, S. Dak. State Coll., Brookings. Milk Dealer, 37, 2: 48, 49, 108-12. Dec., 1947.

A detailed description of the manufacture of cottage cheese by the



rennet method is given. Use of this method results in from 3 to 5 lb. more cottage cheese per 100 lb. of skim milk than is obtained by coagulating the skim milk entirely by the use of starters. The defects of cottage cheese and their causes are discussed. C.J.B.

119. Manufacture of cottage cheese from nonfat dry milk solids. W. H. E. REID AND M. O. MAUGHAN, Univ. of Mo., Columbia. Milk Dealer, 37, 1: 43, 136-138. Nov., 1947.

A new method of making cottage cheese from dry milk solids is described in detail. The method was developed by making more than 100 batches of cheese, using dry milk from 11 different manufacturers, and then checking the process on a commercial scale at 2 dairy plants. The readily available supply of spray process nonfat dry milk solids is used, enabling milk dealers to offer their customers a high-quality cottage cheese at all seasons of the year. C.J.B.

#### CONCENTRATED AND DRY MILK; BY-PRODUCTS

120. Manufacture of cultured buttermilk. A. A. SCHOCK, S. Dak. State Coll., Brookings. Milk Dealer, 37, 1: 140-148. Nov., 1947.

Detailed instructions are given for the manufacture of: (a) plain or cream type buttermilk, made from skim milk or mixtures of whole milk and skim milk having a fat content of approximately 2%; (b) fat granule or fat flake-type buttermilk, which is plain type cultured buttermilk containing butterfat granules; and (c) cultured buttermilk from reconstituted skim milk. Instructions for using the creatine test for determining flavor production of cultures also are given. C.J.B.

#### DISEASES

121. Research and control of mastitis in New York State. H. C. HODGES, Vet. Coll., Ithaca, N. Y. Milk Dealer, 37, 1: 86-89. Nov., 1947.

Surveys made of more than 300 herds and 9,000 cows in New York State since April 1, 1946, show that the percentage of cows infected with pathogenic organisms usually associated with bovine mastitis is well over 35%, with around 25% of the cows infected showing the presence of streptococci. Hemolytic staphylococcus, coliforms, corynebacterium, and occasionally species of *Pseudomonas* also are found. Some herds have practically no infection while others show as many as 90% of the cows with mastitis organisms in their udder secretions.

Early observations indicate that control of bovine mastitis involves three closely associated phases of herd management: (a) Diagnosis, know your cows; (b) prevention, protect cows from exposure to predisposing factors and infection; (c) treatment, use judiciously after proper diagnosis. C.J.B.

122. Scientific discussion on brucellosis at medical milk commission convention. D. T. BERMAN, Univ. of Wis., Madison. Certified Milk, 22, 4: 4. Aug., 1947.

In those areas where cattle predominate in the animal industry, *Brucella abortus* is a major factor in causing human brucellosis. The test and slaughter program has gone about as far as it can go in the eradication of this disease. While calfhood vaccination is beneficial, it alone will not solve the problem; the most important thing which can be expected from calfhood vaccination in infected herds is time in which to exercise the older sanitary procedures of testing, segregation, and elimination, with the eventual aim of the development of brucellosis-free herds.

More research work is needed on the value of repeated vaccinations and also on improving the means of distinguishing infected reactors from those reacting because of vaccination with strain 19. Further research may show that it is possible to keep herds at a high level of resistance by annual vaccination. However, an important factor to consider before adopting re-vaccination or vaccination of adults is the possibility of establishing strain 19 in the udder of the lactating cow and, should this occur, its potential significance as a source of brucellosis in humans. W.S.M.

123. Ketosis (Acetonemia) in dairy cattle. J. C. SHAW, Univ. of Maryland, College Park. Certified Milk, 22, 7: 15, 16. Nov., 1947.

Ketosis, an ailment often mistaken for milk fever, is caused by a decrease in the amount of sugar in the blood rather than a decrease in blood calcium. It occurs most frequently in high-producing cows in late winter and early spring. Experimental evidence indicates: That a restricted feed intake before or after calving, or both, is probably only one of the factors causing ketosis; that ketosis is not due to a deficiency of vitamin A in the feed; and that massive doses of methionine, thiamine and other B vitamins, when administered both intravenously and orally, have no beneficial effect. The recommended treatment is the injection of glucose directly into the venous blood, supplemented by the feeding of some form of soluble sugar. When the case is difficult to diagnose or when ketosis and milk fever both are involved, it is wise to inject calcium gluconate followed by a straight glucose solution. The urine test alone is not infallible in diagnosing ketosis; it should be used in conjunction with information on the symptoms of ketosis. W.S.M.

## FEEDS AND FEEDING

124. Rumen digestion studies. I. A method of investigating chemical changes in the rumen. E. B. HALE, C. W. DUNCAN, AND C. F. HUFFMAN. Mich. State Coll., East Lansing. J. Nutrition, 34, 6: 733-745. 1947.



Methods of determining digestion coefficients and rate of digestion in the rumen are discussed. The bases of calculations for such studies are treated in detail.

R.K.W.

125. Rumen digestion studies. II. Studies in the chemistry of rumen digestion. E. B. HALE, C. W. DUNCAN, AND C. F. HUFFMAN. Mich. State Coll., East Lansing. *J. Nutrition*, 34, 6: 747-758. 1947.

Rumen digestion was studied in cows with fistulas. During the first 6 hr. following feeding, the predominant digestion was of proteins and carbohydrate. During the second 6 hr. following feeding, there was a rapid digestion of cellulose with digestion of protein and carbohydrate continuing at a rate paralleling that of cellulose digestion. Rumen digestion was complete after 12 hr. Average coefficients for rumen digestion of roughage 12 to 14 hr. following feeding were: dry matter 48.4, protein 59.6, nitrogen-free extract 65.2, crude fiber 27.2, cellulose 43.4, other carbohydrates 83.0 and lignin 3.1%. Synthesis of fatty acids in the rumen was demonstrated. Apparently there is rapid removal of the fatty acids following their synthesis.

R.K.W.

126. Mineral metabolism studies in dairy cattle. I. The effect of manganese and other trace elements on the metabolism of calcium and phosphorus during early lactation. J. T. REID, K. O. PFAU, R. L. SALSURY, C. B. BENDER, AND G. M. WARD, N. J. Agr. Expt. Sta., SUSSEX. *J. Nutrition*, 34, 6: 661-676. 1947.

The effects of Ca, Mn and other mineral supplementation upon Ca and P balance were studied during the first 5 months of lactation of 12 cows. A ration of grain, corn silage, and timothy-clover hay was supplemented with either  $\text{CaCO}_3$ ,  $\text{CaCO}_3$  plus  $\text{MnSO}_4$ , or Mico, which was a commercial calcium source containing several trace elements. Phosphorus balances were not affected appreciably by these supplements. Manganese sulfate supplementation decreased Ca retention, while Mico increased Ca retention.

R.K.W.

127. Mineral metabolism studies in dairy cattle. II. Effect of calcium and manganese and other trace elements on the metabolism of lipids during early lactation. G. M. WARD AND J. T. REID, N. J. Agr. Expt. Sta., Sussex. *J. Nutrition*, 35, 2: 249-255. 1948.

A study of effects of Ca, Ca plus Mn, and Ca plus Mn and other trace element supplementation upon fecal excretion of lipids was made with dairy cows. All groups of cows excreted similar proportions of total lipid intake, as measured by acid hydrolysis and chloroform extraction. However, calcium-supplemented animals were observed to eliminate lesser



proportions of ether extract. This was attributed to a greater amount of calcium soap formation in cows fed the calcium supplements, these soaps not being measured because of their insolubility in di-ethyl ether.

These data indicate the possibility of error in crude fat digestion determinations.

R.K.W.

128. Thiamine deficiency in the calf. B. C. JOHNSON, T. S. HAMILTON, W. B. NEVENS, AND L. E. BOLEY, Univ. of Ill., Urbana. *J. Nutrition*, 35, 2: 137-145. 1948.

Thiamine deficiency in the young calf was demonstrated. This deficiency was characterized by weakness, incoordination of legs, and convulsions. In addition, some calves exhibited scouring, anorexia and dehydration. The symptoms responded to thiamine therapy except in cases of severe dehydration.

R.K.W.

129. How to make up an economical dairy ration. ANONYMOUS. U. S. Dept. of Agr., Bureau of Dairy Industry, Publication BDIM-Inf-57. 4 pp. 1947.

A procedure based upon 4 different qualities of roughage, 3 levels of digestible protein in the concentrates and the cost per ton of total digestible nutrients in the various concentrates is outlined, and examples of the necessary calculations are given. Four tables, including one giving a schedule of suggested concentrate feeding for cows producing at different levels, are given to provide data necessary for the calculations of the most suitable ingredients under different conditions.

F.E.N.

130. More milk from your forage crops. R. E. HODGSON, U. S. Dept. of Agr. *Certified Milk*, 22, 7: 4, 5. Nov., 1947.

When alfalfa-Ladino clover forage was made into wilted grass silage, 7.2% more dry matter and 26.4% more protein were obtained than when curing the crop as hay. The silage dry matter at the time it was fed later in the winter contained 10 times more carotene than did the hay. This difference in feed carotene showed up in the milk produced by the cows.

The advantages of the wilting method for making grass silage over other methods are: low moisture content; freedom from obnoxious odors; highly palatable, so cows will consume more dry matter; little or no drainage from silo; less expensive and troublesome to make. Harvesting experiments showed no particular difference in the amount of labor and field machinery time required to harvest a ton of dry matter as silage or as hay. Under average conditions grass silage should not be considered a substitute for corn silage but rather as a substitute for part or all of the hay and feed as such, along with corn silage.

W.S.M.

131. Some observations on beef cattle affected with generalized edema or anasarca due to vitamin A deficiency. L. L. MADSEN AND I. P. EARLE, Bureau of Animal Ind., Beltsville. *J. Nutrition*, 34, 6: 603-619. 1947.

During the period of July, 1941, to Dec., 1946, 651 beef carcasses were condemned for generalized edema or anasarca by Federal meat inspectors. This condition was observed to occur in cattle after a long period in dry lot when the diet consisted of corn and a low-carotene roughage, such as oat hay or straw.

Cases of anasarca were produced experimentally by feeding carotene-deficient rations. Alfalfa hay was effective in curing the condition in the field. Blood studies also showed similarity between experimental and field cases of anasarca; such condition apparently is due to vitamin A deficiency. R.K.W.

132. Comparison of vitamin A liver storage following administration of vitamin A in oily and aqueous media. A. E. SOBEL, M. SHERMAN, JACQUELINE LICHTBLAU, SELIG SNOW, AND B. KRAMER. Jewish Hospital of Brooklyn, N. Y. *J. Nutrition*, 35, 2: 225-238. 1948.

Liver storage, in rats, of vitamin A from oily and aqueous media was studied. Vitamin A liver storage was greater when the vitamin A source was dispersed in water than when vitamin A was in the oily medium. These data indicate importance of considering the nature of diluent for biological evaluation of vitamin A. R.K.W.

#### FOOD VALUE OF DAIRY PRODUCTS

133. Nutritional studies on milk fat. III. The effect of the treatment on milk fat with certain solvents on the growth of young rats. E. L. JACK AND E. B. HINSHAW, Univ. of Calif., Davis. *J. Nutrition*, 34, 6: 715-724. 1947.

Pentane (Skelly-solve A) contains an impurity which lowers the growth-promoting properties of milk fat. Treating the pentane with fuming sulfuric acid removes this deleterious action. A -53° filtrate milk-fat fraction prepared with the purified pentane produced greater growth in young rats than the original fat. Previously a -53° fraction milk fat, obtained by using unpurified pentane, gave slightly less growth than untreated milk fat. R.K.W.

#### ICE CREAM

134. Control of raw materials and partly finished products in the ice cream plant. A. H. BAYER, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 43, 12: 44. Dec., 1947.



The use of daily factory records is desirable and makes possible the control of profits affected by production. These records are current, and they point out inefficiencies in operation, possible theft, waste in labor and operation, and other conditions that could not otherwise be observed. Factory records account daily for materials and products used, for materials and products sold and for those remaining in inventory. Simple forms worked out by the production and accounting departments will make possible the collection of needed information in a neat, orderly and uniform manner. Standards for each operation, such as units of production per man hour in the mixing, freezing, packaging, novelty and other operations, may be set up.

A list of the factory records which may be used includes: a daily report of mix made, a monthly mix report, a daily freezing report, a daily report of operations, a withdrawal slip for charging out products from the hardening room, a report showing products put into the hardening room, a hardening room inventory report, and a planning report showing ingredients needed, finished product desired, hours and people needed for the job.

Records properly kept will provide information on cost of products, proper selling prices, proper planning of production and efficient plant operation, point out possible theft, and aid in quality control. They are a necessary part of any manufacturer's business. W.H.M.

135. Evaluating the flavor of ice cream. D. V. JOSEPHSON, Dept. of Dairy Technol., Ohio State Univ., Columbus. *Ice Cream Trade J.*, 43, 12:48. Dec., 1947.

Attempts were made to determine consumer preferences with respect to the sugar, butterfat and serum solids content of ice cream. Groups of students tested over a period of 3 yr. indicated that 75% consistently preferred ice cream containing 14 to 15% sugar, 60% preferred ice cream containing 12% fat, and the percentage preference increased for serum solids as the amount was increased from 8 to 13%.

In the evaluation of flavor, three functions are used: (a) taste (gustatory response), which is the result of certain organs on the tongue; (b) touch (tactual response), which is the result of the stimulation of other organs on the tongue; and (c) smell (olfactory response), which comes from the chemical stimulation of the olfactory cells located at the base of the nasal cavities. People do not have the same degree of development or refinement in their taste functions. Other complications are involved, such as temperature of samples, size of sample, eating habits of the individual, age and rate of recovery of taste mechanism between samples. In most judging work a panel of 3 to 5 judges is used. This system has certain weaknesses, such as time of judging, number of samples tasted,



presence of a dominant figure who sets standards for the group, and wide range of numerical points assigned to flavor scores on all factors, which cause results from the panel to vary from the results which might be obtained from an average consumer.

Suggestions are offered for improving present methods of evaluating flavor of ice cream. The relative sensitivities to basic, abnormal and deteriorative flavor qualities of each member of the panel should be determined. If numerical ratings are used, a maximum range of 5 or 6 points should be employed. Each taste observer should have complete freedom in expressing his judgment. A standard and uniform terminology for describing the flavor and texture qualities of ice cream should be established. Judging sessions should be planned so that observers will have ample time, preferably before meals. Avoid disturbance or noise. Evaluate the product in terms of consumer preference, if known. Remove all identification marks from samples. The individual who sets up the samples and takes the data should not be a member of the panel. W.H.M.

136. Utilizing the true lactic acid content to indicate ice cream quality. I. A. GOULD AND F. A. POTTER, Univ. of Maryland, College Park. Ice Cream Trade J., 43, 12: 46. Dec., 1947.

Some method other than the titration procedure is needed for the determination of the actual lactic acid content of ice cream. In recent years the Hillig colorimetric method for the determination of lactic acid has been developed in the laboratories of the Pure Food, Drug, and Cosmetic Administration and has been used to indicate the quality in dry milk solids; the procedure has been accepted by the Association of Official Agricultural Chemists. The Hillig method was applied to ice cream.

The method used was essentially the A.O.A.C. method with modifications. Of 10 samples of ice cream tested, 6 contained less than 13 mg. % of lactic acid. No relationship was found between the lactic acid content and titratable acidity and pH of the mixes. The method was found to be accurate generally within 2 mg.%. Normal values were increased somewhat by addition of certain flavoring components but not sufficiently to invalidate the use of the lactic acid method as a quality test. W.H.M.

137. The use of liquid sugar in ice cream. H. G. DUNLAP, H. P. Hood and Sons, Inc., Providence, R. I. Ice Cream Trade J., 43, 12: 54. Dec., 1947.

The Hood Co. has been using liquid sugar for 17 yr.; economy and quality problems were responsible for the practice. A saving of 30 cents per cwt. in purchase price and 15 cents net in handling costs has resulted. No trouble was experienced with fermentation; however, it was found necessary to keep the Brix at 67° in order to prevent crystallization. Sirup

is trucked as far as 100 miles and rail tank cars are used for shipping 240 miles to a condensery. Storage is not much of a problem but requires planning on the estimated amount needed. Meters and an auxiliary 100-gallon tank are used to measure the sirup; however, the meters are not as satisfactory as the calibrated tank.

W.H.M.

138. The Bowman dairy program for building "dry stop" volume. H. A. QUITTER, Bowman Dairy Co., Chicago, Ill. Ice Cream Trade J., 43, 12: 42. Dec., 1947.

A dry stop setup for improving the appearance of dealers' stores and increasing profits has been developed. The setup consists of a 5-ft. tile board back bar, with a 4-ft. fluorescent light behind a canopy. In the center of the back bar is a permanent flavor board and on either side 2 frames in which advertising may be displayed. Electric connections for mixers and hot cups are provided. This bar is hung on the wall about 30 in. to the rear of the ice cream cabinet and a counter is fitted on top of the cabinet. A specially constructed sirup tray holding 4 2-qt. shallow pans is fitted into one of the openings of a wooden flip-lid cabinet. A dipper well also is connected to the cabinet. Through an arrangement with a paper cup company, the ice cream dealer is furnished with a combination package consisting of 300 4-oz. cups for a 10 cent sundae, 300 8-oz. cups for a 20 cent sundae, 300 16-oz. malt cups, 300 banana split boats, and 2 metal mixing collars for mixing malts and milk shakes in the 16-oz. cups.

The salesman is paid a bonus of 1.5 cents per gallon for every gallon each dealer increased over the previous year's business and a penalty of 2 cents for every gallon under the previous year's business.

In attracting people to ice cream stores, animated displays are to be preferred over the paper window displays. The fountain is placed in the brightest spot in the store and pictures of the various items sold are displayed at the fountains. Trained, neat, courteous, and efficient help is employed. Cleanliness at the fountain, good quality toppings and uniform servings help increase fountain business.

W.H.M.

## MILK

139. Proper store differentials. R. C. CRABB, General Ice Cream Corp., Schenectady, N. Y. Milk Dealer, 37, 2: 42, 80-82. Dec., 1947.

Based on experiences in 4 markets in upper New York State and in New England, the author points out that a grocery store owner today is realizing the same penny profit per quart of milk as he did years ago when milk sold for several cents a quart less. Many grocers treat milk as a poor relation and do not realize that milk can pull more customers into their stores than any other item, with the possible exception of bread. Some un-



favorable factors involved in milk handling are: the low profit per unit of sale, the refrigeration required, the handling of empty bottles and the supplying of a bag. However, milk has the first or second highest turnover of any item in the store. Being perishable, milk attracts customers to the store daily. Surveys show that 86% of people returning bottles make purchases averaging 66 cents. No money is tied up in inventory, as with canned goods. No space is needed for storage of extra inventory either in the basement, back room, or in an outside rented warehouse.

The milk dealer should aid the grocer to increase his milk sales by urging him to keep his milk displayed, preferably in a self-service case, and by using sales promotional material in his store. The dollar sales should be built up so that the per cent of profit will be of secondary importance. Through a process of continually talking in terms of dollars and cents rather than per cent of profit, the grocer's apparent indifference to milk sales can be broken down.

C.J.B.

140. Fat variation in milk tests. T. M. BINNEY, Purdue Univ., Lafayette, Ind. *Am. Milk Rev.*, 8, 8: 52-53. 1946.

Factors which may be influential in causing variation in fat tests are: breed of cow, individuality of cows, feed, season, weather, stage of lactation, age, time interval between milkings, completeness of milking, health of the cows, exercise, management, environment, farm skimming practices, and conditions during transportation of the milk. During the month of May composite herd tests from one herd varied from 4.7 to 5.8%; daily variations on composite samples ranged from 0 to 0.7% fat. In another herd, during the same month, fat tests from composite herd samples ranged from 5.1 to 5.9% and daily variations ranged from 0 to 0.6% fat. P.S.L.

141. Consideration on the keeping quality of pasteurized milk. L. H. BURGWARD AND D. V. JOSEPHSON. Ohio State Univ. *Am. Milk Rev.*, 8, 1: 26-47. 1946.

Regular grades of pasteurized milk were found to stay sweet for 7 to 28 days under conditions which might normally be imposed upon it by consumers. Milk was considered sweet until the acidity increased by 0.03%. The average keeping quality under all conditions studied was greater than 12 days. The initial bacterial counts did not always indicate potential keeping quality of the milk. On the average, the low count milk had very slightly better keeping quality than the higher count milk. There was no appreciable increase in bacterial count for the first 4 days. After 4 days, the psychrophilic bacteria increased rapidly and invariably numbered over a hundred million at the time the milk soured. No flavor change was noted in any of the samples before 4 days. The half pints held below 40° F. developed a flavor change at an average of 12.1 days.



The average vitamin C content was 10.5 mg. per l. in the fresh milk which had not been unduly exposed to light or room temperature. The quarts exposed for 2 hr. at room temperature contained 8.5 mg. per l. after exposure. The vitamin C in 4 lots had been reduced from 7.4 to 0.6 mg. per l. in 2 days even without exposure, probably due to iron or copper contamination. The milk from the other 4 lots not exposed had an average of 13.7 mg. of vitamin C per l. in fresh milk, 9.7 mg. at 2 days, and 6.4 mg. at 4 days. The riboflavin content of all lots of milk was practically the same, with an average of 1.57  $\gamma$  per ml. Practically none was lost upon storage, the average being 1.55  $\gamma$  per ml. at the point of souring. P.S.L.

142. A test for the milk plant as an added protection. A. V. MOORE AND G. M. TROUT. *Am. Milk Rev.*, 8, 8: 22-24. 1946.

Five series of homogenized and non-homogenized milk to which various increments of raw milk were added were observed for the development of rancidity, changes in titratable acidity and pH, and reaction to the phosphatase test. At a storage temperature of 40° F., rancidity developed in samples of homogenized milk which were contaminated with raw milk, and the titratable acidity increased, along with a decrease in pH. Detection of raw milk contamination was shown by the phosphatase test when 0.5% of raw milk was present but was questionable when only 0.1% of raw milk was present. It was found necessary to have at least 4% of raw milk present to detect contamination in homogenized samples at the end of 24 hr. upon the basis of rancidity as judged by taste and smell. Extraction of the phenol with butyl alcohol was found necessary in order to make the results of the phosphatase test accurate for homogenized milk. P.S.L.

143. Technical considerations in leveling milk production. L. C. CUNNINGHAM, Cornell Univ. *Am. Milk Rev.*, 8, 9: 48-54. 1946.

Factors in leveling fall milk production include consumer demand for milk, seasonality of milk prices received by farmers, and dairy farm organization and income. Seasonality of milk production is increasing in importance due to an increase in consumer demand for fluid milk. In New York state, the cost of producing milk is 30% lower in the summer and 25% higher in winter than the average cost for the year.

To facilitate the study of seasonal patterns in milk production, dairy farms in each of 7 areas in New York were classified as even dairies, spring dairies, summer dairies, and winter dairies. Even dairies included those in which daily deliveries in the lower quarter were at least 70% of deliveries in the higher quarter. Spring dairies had their highest quarter in April-June, summer dairies in July-Sept., and winter dairies in Jan.-March. Farm records used were not all for the same period. Herds

tended to be larger in the even dairies, but generally there was little relationship between seasonal production and herd size.

Production per cow definitely was higher in the winter dairies than in spring dairies, the average difference ranging from 600 to 2,000 lb. per cow, depending on the area. Production in summer dairies was the lowest of all types. The labor force is used more efficiently in winter dairies. The output of milk per man in winter dairies exceeded that found in spring dairies in all areas; in 3 particular areas the amount was 0.5 can daily per man. The year-round cost of producing 100 lb. of milk was lower in winter dairies than in spring dairies, the difference ranging from 6 to 20% in various areas. The average difference in cost was 30 cents per cwt.

Winter dairies were more profitable than spring dairies, the difference in income ranging from \$300 to \$1,200, depending upon total income. Differences between income from winter and even dairies were insignificant. Summer dairies were the least profitable. The relatively higher incomes in winter and even dairies were due primarily to higher milk production per cow and per man and to a considerably lesser extent to higher milk prices in the fall. An increase in fall and winter milk production to meet consumer demand is in line with sound dairy farm management.

P.S.L.

144. Paper bottle. F. D. PALMER. (Assigned to F. D. Palmer, Inc.) U. S. Patent 2,435,155, Jan. 27, 1948. Official Gaz. U. S. Pat. Office, 606, 4: 642. 1948.

A rectangular-shaped paper container is described suitable for distributing milk and other liquids. For dispensing the product in a sanitary manner, an opening and pouring spout arrangement is provided which is sealed at time of filling and which cannot again be resealed once it is opened.

R.W.

145. Refrigeration for the milk bottling plant. L. BUEHLER, JR., Creamery Package Manufacturing Co., Chicago, Ill. Milk Dealer, 37, 1: 92-98. Nov., 1947.

A refrigerating plant must be of sufficient size, reliable in operation, require a minimum of attention, and be simple enough that the operator need not be highly skilled. The advantages and disadvantages of the direct expansion system in which the refrigerant, such as ammonia, is used directly in the milk cooler, ice cream freezer or room cooling coils to extract the heat from the product and the chilled water system, with and without ice storage, are discussed. The choice would depend upon the peculiar requirements of any specific job.

C.J.B.



146. Sales, expenses, and profits of six leading milk companies in the New York-New Jersey Metropolitan Area. L. SPENCER, Cornell Univ., Ithaca, N. Y. Milk Dealer, 37, 1: 45, 46, 117-122. Nov., 1947.

During the 6 yr., 1941-1946, 6 of the principal companies distributing milk in the New York-New Jersey Metropolitan Area sold \$159 million worth of products yearly, their net profit amounting to 1 cent per dollar of sales. These companies had smaller profits per dollar of sales and per dollar invested by the owners than any of a number of representative groups of companies in different kinds of business throughout the country. Companies manufacturing drugs and soap averaged 8.4 cents per dollar of sales; 15 dairy companies doing business in various parts of the country, including operations in ice cream, evaporated milk and cheese, earned 2.5 cents per dollar of sales in this 6-yr. period.

The return on the owners' investments in the 6 milk companies for the period was 3.3%. This rate was lower than that for any of 17 important groups of companies whose financial statements are made public. During the period studied the dollar sales of the 6 milk companies increased 67% while the amount paid for milk and other products purchased went up 87%. These gains were due mainly to the general advance in prices rather than to growth in physical volume of business. Product cost amounted to 55% of sales in 1941 and 61% in the year ending June 30, 1947, while the gross spread between sales and product cost became 44% greater.

A little more than 12 cents a quart is paid by the dealer for his milk supply at country receiving plants. Operating costs of all kinds on milk delivered to the consumer's doorstep come to nearly 10 cents; of this amount, more than 6 cents goes for delivery service. The dealer's profit is perhaps as much as 0.5 cent a quart before taxes are paid. This is equivalent to 1.5 cents per dollar sales. Of the 20 cents a quart paid by consumers for a quart bottle of standard milk at stores, 2 cents is retained by the storekeeper and 18 cents goes to the milk dealer. His operating costs total about 5.6 cents, including 2.4 cents for delivery service. Under present conditions his net profit after all taxes is no more than 0.2 cent a quart, or 1 cent per dollar of sales.

C.J.B.

## SANITATION AND CLEANSING

147. A comparison of the cleaning of square and round milk bottles under regular commercial conditions. T. V. ARMSTRONG AND L. H. BURGWARD. Ohio State Univ. Am. Milk Rev., 8, 1:34-37. 1946.

Washed bottles were obtained from 7 different makes of washers in 9 different plants during the months from June through Sept., 1945. Some of the washers were able to accommodate the square bottle without any



change, while others had to undergo adjustments. The dirty bottles used were the regular run of bottles as returned to the plant or brought from another plant. The procedures used in making the counts were those outlined in the Eighth Edition of "Standard Methods for the Examination of Dairy Products". After plating, the bottles were examined under a daylight lamp and the presence of specks or streakiness was noted. The bottles then were filled with milk and again examined for specks and streaks.

Counts in excess of 1,000 per bottle were obtained on 4.3% of the square quart bottles and 7.6% of the round bottles. Specks of dirt were found in 1.1% of the square bottles and 5.3% of the round bottles. Of 15 different lots of one-half pint bottles, counts in excess of 250 per bottle were obtained on 9.6% of the square bottles and on 8.6% of the round ones. No visible dirt was observed in any of the square bottles of this size, while visible dirt was observed in 8.6% of the round ones. These studies and data show that there is no difference in the commercial practicability of cleansing and sterilizing the returnable square milk bottles and the conventional round bottle in typical dairy soaker equipment. P.S.L.

#### MISCELLANEOUS

148. Waste saving and disposal. O. W. SONBORG AND H. J. STEFFEN, Chicago, Ill. *Am. Butter Rev.*, 9, 7: 40-43. 1947.

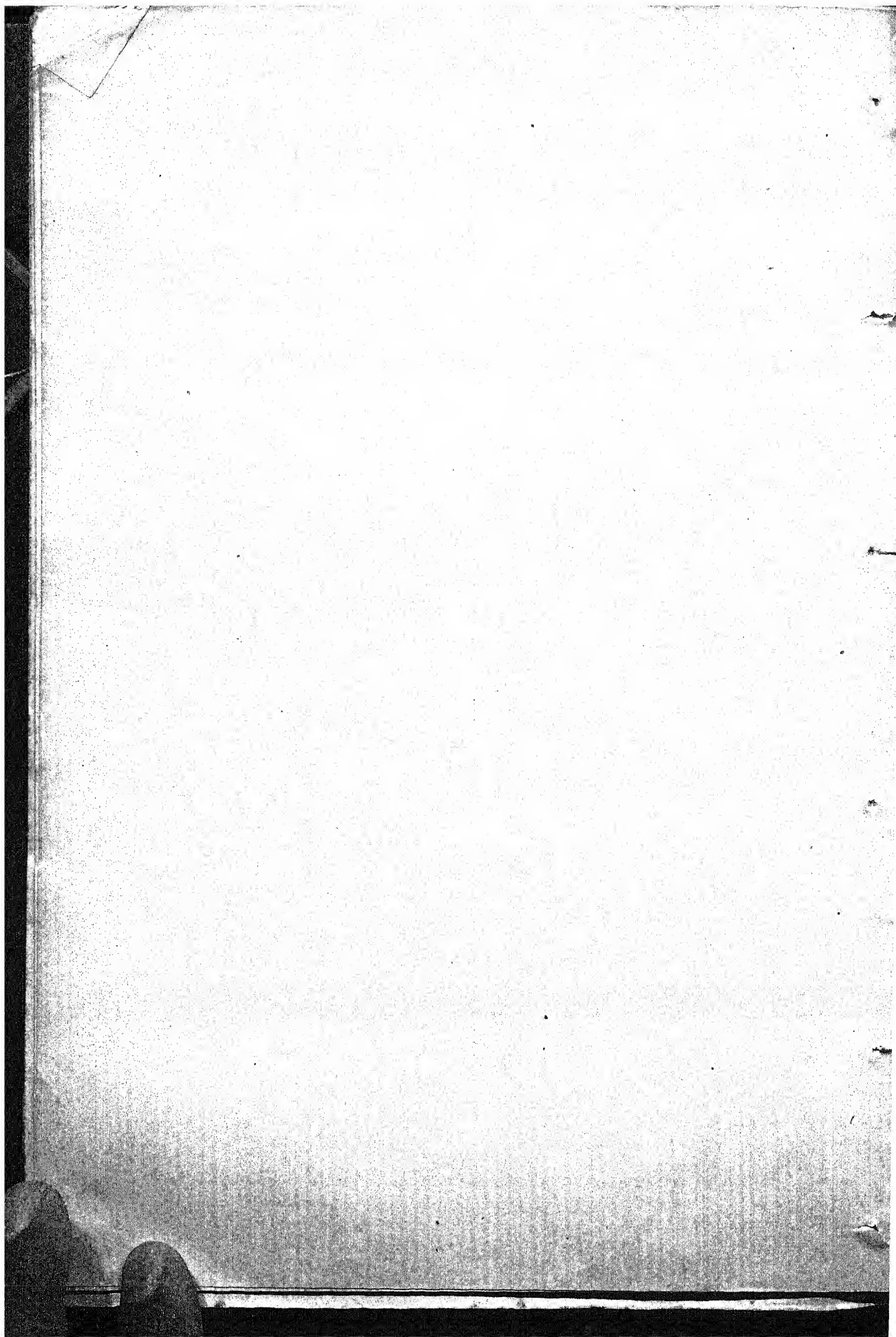
Dairy wastes can be reduced by drip savers on can washers, steaming cream cans, pre-rinse of milk cans, electronic liquid level controls on vats, accurate temperature control on plate coolers, standby power equipment, adequate storage tanks, prevention of leakage, prevention of foaming, saving of buttermilk for livestock feed, and provision of facilities for processing skim milk. Some waste from rinsings and wash water is unavoidable. When the amount of waste is small, a septic tank may be sufficient as a means of disposal. Another elementary treatment is accomplished through the use of an aerated equalizing tank. Trickling filters may be used to give more complete treatment when necessary. The filters are composed of a coarse medium, such as gravel or coke, and the sewage allowed to flow over this medium at such a rate as to allow free passage of air. P.S.L.

149. The training of young men for the dairy industry. R. B. STOLTZ, Dept. of Dairy Technol., Ohio State Univ. *Milk Dealer*, 37, 2: 86-90. Dec., 1947.

Thirty-five years ago the universities and colleges were not attempting to turn out dairy technologists or managers for dairy plants; they were training buttermakers, ice cream makers, and other dairymen—who knew how to produce milk and manufacture it into dairy products. Today the attempt is to train executives whose foundation will rest upon chemistry,

mathematics, physics, bacteriology and accounting. The Ohio State curriculum today does not require students to take agricultural subjects such as entomology, farm crops, soils, horticulture and geology. Instead, they are required to take applied subjects, such as speech, marketing, advertising and salesmanship.

The author also discusses the training of students after they have finished the university. An employer should take the men when they are available and not wait until he needs them. Too much should not be expected of the graduates, as they still are boys. The men should be contacted to demonstrate management knows they exist, given responsibility, encouraged to make progress, given a change of work, shown that management has confidence in them, and should be paid what they are worth. C.J.B.





## ABSTRACTS OF LITERATURE

### BOOK REVIEW

150. **Legal aspects of milk sanitation.** JAMES A. TOBEY. 133 pp. \$5.00. Milk Industry Foundation, Washington, D. C. 1947.

This is the second edition of a book dealing with the legal responsibilities of milk producers and processors and distributors of milk and milk products. It is concerned with responsibilities relating to public health rather than to price fixing. The sources and nature of laws and regulations relating to milk sanitation are described. Many court decisions and opinions are cited which show the validity of various laws and regulations. The book is of value as a source of information concerning the extent and limitations of responsibilities of handlers of dairy products insofar as these products are related to public health. Chapter headings include the following: I. Reasons for the Public Control of Milk, II. The Sanitary Regulation of Milk by the State, III. Municipal Control of Milk, IV. Licenses and Permits, V. Standards for Milk and Milk Products, VI. Inspection, Sanitation and Seizure of Milk, VII. Tuberculin Testing and the Health of Dairy Cattle, VIII. Pasteurization, IX. Containers for Milk, and X. Liability in Connection with Dairy Products.

M.P.B.

### BUTTER

151. **Controlling the composition of butter.** R. W. BROWN. Can. Dairy Ice Cream J., part 1, 26, 11: 33. Nov., 1947; part 2, 26, 12: 23. Dec., 1947.

The composition of butter is controlled because it must conform to state and federal standards, operating losses must be reduced to a minimum, and butter must retain its weight and moisture to avoid danger of short weighting. A knowledge of the factors that affect overrun and overrun control is necessary in order to control the composition of butter. Calculations are given for overrun, theoretical overrun, composition overrun, churn overrun, creamery overrun, accuracy of testing cream, losses of fat in buttermilk, overrun of unsalted butter, and water addition.

H.P.

152. **Die Bedeutung des Acetoin und Diacetyls für das Aroma von Rahmsäurungskulturen.** (The importance of acetoin and of diacetyl upon the aroma of butter cultures.) J. RODENKIRCHEN. Die Milchwissenschaft, 2, 8: 329-335. Aug., 1947.

Butter cultures grown in heat-treated skim milk (75° C. for 30 min.) and held for 3 days varied in intensity of the acetoin-diacetyl reaction when examined daily. The intensity of the acetoin-diacetyl reaction did not correlate with presence or absence of chains of organisms or with the

quality of flavor produced. Generally the intensity of the reaction of the individual cultures remained at the same level for 3 days when cultures were incubated at 20° C. for 20 hr., followed by a lower holding temperature.

The acetoin and diacetyl formation was found to be independent of the pH of the starter cultures. Also, the amount of these compounds formed was not influenced by the available oxygen supply.

Higher incubation temperatures (50–60° C.) increased the production of acetoin and of diacetyl. Reheating of the cultures resulted in a more distinct acetoin-diacetyl reaction. Growth of butter cultures in whole milk generally resulted in higher production of acetoin and diacetyl than growth of the same cultures in skim milk. The author states that the acetoin-diacetyl reaction according to Vas Criszár is not suitable for objective measurements of the quality of flavor in butter cultures. I.P.

### CHEESE

153. A rapid method for determining extraneous matter in Cheddar cheese. E. G. HOOD. *Can. Dairy Ice Cream J.*, 27, 1: 19–20. Jan., 1948.

A 15-g. sample of Cheddar cheese is placed in a clean Waring Blendor jar of 1,000-ml. capacity, and 200 ml. of 10% sodium citrate solution at a temperature of 75 to 80° C. is added. After 2 minutes of disintegration, the sample is transferred to a funnel under suction, using a lintine filter disc. The advantages over the older method for removing the extraneous matter from Cheddar cheese are: (a) a smaller sample can be used (15 g. compared to 227 g.); (b) hot citrate solution is used for more rapid disintegration; (c) the sample can be obtained from one cheese plug, preventing damage to the cheese; and (d) the method is much faster. The test can be carried out in 4 minutes, an added incentive for extending the use of the method to an educational program or to regulatory control. H.P.

154. Apparatus for use in the centrifugal separation of serum from cheese constituents. G. J. STREZYNSKI. (Assigned to DeLaval Separator Co.) U. S. Patent 2,436,498, Feb. 24, 1948 (12 claims). *Official Gaz. U. S. Pat. Office*, 607, 4: 683. 1948.

The details are given for a continuous type separator bowl which may be used for removing serum from a standardized coagulated dairy product, thus producing a curd of such moisture content that it may be used for cream cheese. R.W.

### CHEMISTRY

155. Lactoflavin-reduktase. (Riboflavin-reductase.) M. E. SCHULZ. *Die Milchwissenschaft*, 2, 3: 152–160. March, 1947.

The role of riboflavin as an oxidation-reduction indicator in milk and

its relative position with respect to other redox systems are pointed out. The usefulness of riboflavin as an indicator of oxygen tension in growing cultures of *S. lactis* in milk is demonstrated and the role of riboflavin in the formation of diacetyl in cream ripening is discussed. I.P.

156. The use of the sodium-chlorine relationship for the detection of sodium neutralized non-fat dry milk solids. W. HORWITZ, Food and Drug Administration, Federal Security Agency, Minneapolis, Minn. J. Assoc. Offic. Agr. Chemists, 31, 1: 121-124. 1948.

The sodium and chlorine contents of 23 samples of sodium-neutralized and 81 samples of normal or non-sodium-neutralized dry skim milks were determined. The ratio of sodium to chlorine in the normal samples was 0.47, with a standard deviation of 0.03. If the per cent sodium is plotted as the abscissa against the per cent chlorine as the ordinate, all of the sodium-neutralized samples lie to the right of the line represented by the equation: % Na = 0.62% Cl - 0.10. All of the normal or non-sodium-neutralized samples lie to the left of this line. F.J.B.

157. Serum methods for added water in milk. D. J. MITCHELL AND G. G. FRARY, State Chemical Lab., Vermillion, S. D. J. Assoc. Offic. Agr. Chemists, 31, 1: 124-127. 1948.

The mean value of 3 serum methods (acetic serum, sour serum, and copper serum) did not indicate added water until more than 10% added water was present. The copper serum method was the most rapid from the standpoint of preparation of the serum and gave a narrow range of readings. The authors state that the cryoscopic method should be used to check the serum methods whenever added water is indicated, since the method is rapid, accurate and most reliable. F.J.B.

158. The determination and identification of lactic and succinic acids in foods. H. V. CLABORN AND W. I. PATTERSON, Food and Drug Administration, Federal Security Agency, Washington, D. C. J. Assoc. Offic. Agr. Chemists, 31, 1: 134-139. 1948.

A method is outlined for the determination of lactic acid in liquid whole or skim milks and in dried whole or skim milks. Detailed procedures are given for preparation of the sample, preparation of the sodium salt of lactic acid, preparation of the partition column, isolation and identification of lactic acid. F.J.B.

159. The determination of free tryptophane in milk, cream and butter. R. E. DUGGAN, Food and Drug Administration, Federal Security Agency, New Orleans, La. J. Assoc. Offic. Agr. Chemists, 31, 1: 151-162. 1948.

A method is described for the extraction and measurement of the free



tryptophane in milk, cream and butter. Investigations show that negligible quantities of free tryptophane are present in normal sweet cream and milk. The amount of free tryptophane in milk and cream increases with age if the products are held under conditions conducive to bacterial and enzymatic activity. The amount of free tryptophane in butter depends upon the free tryptophane content of the original cream. F.J.B.

160. Residual chlorine in milk after the addition of hypochlorite. F. B. MORELAND, Kansas State Board of Health, Topeka. J. Assoc. Off. Agr. Chemists, 30, 4: 655. 1947.

Sodium hypochlorite was added to milk in concentrations ranging from 1,000 to 5 p.p.m. and the milk allowed to stand at room temperature for varying periods of time. At certain intervals, the amount of residual chlorine was determined by titration with thiosulfate and by the Rupp test (A.O.A.C. Methods of Analysis, 6th ed., 1945, p. 317). Data show that the residual chlorine of the milk decreased rapidly. At a chlorine dosage of 100 p.p.m. in the milk, the value dropped to zero in about 15 min. However, the Rupp test continued positive long after the residual chlorine reached zero. The author states that the term "available chlorine" as used in the heading of the table interpreting the reactions to the Rupp test is apt to be misleading, since the values given are for the amount of available chlorine which is present at the instant of its addition to the milk rather than when the Rupp test actually is performed, perhaps much later. Tests also were made on milk containing 100 to 5 p.p.m. available chlorine and held in a refrigerator. F.J.B.

161. Das Fettverderben und seine Bedeutung für Wirtschaft und Leben. (The deterioration of fat and its importance in economy and in life.) H. SCHMALFUSS. Die Milchwissenschaft, 2, 8: 335-347. Aug. 1947.

The review covers 726 publications and patents issued between 1893 and 1944, dealing with fat deterioration. The author distinguishes between 5 main types of fat deterioration, namely, tallowiness, peroxide formation, hydrolysis, ketone formation and aldehyde formation. I.P.

162. Biophysical studies of blood plasma proteins. VIII. Separation and properties of the gamma globulins of the sera of normal cows. E. L. HESS AND H. F. DEUTSCH, Univ. of Wis. J. Am. Chem. Soc., 70, 1: 84-88. 1948.

The conditions of separation, yield and some physical properties of normal bovine serum gamma globulins are reported. The procedure recovers 85% of the gamma globulins, which can be further separated into fractions with varying electrophoretic mobilities. As a result of this and

previous studies, it is anticipated that practical and economical ethanol fractionation methods for the removal of antibody from hyperimmune sera soon will be practical.  
H.J.P.

163. Crystalline pepsin-resistant protein from skeletal muscle. J. BOURDILLON, N. Y. State Dept. of Health, Albany. Arch. Biochem., 16, 1: 61-68. 1948.

A crystalline protein-like substance characterized by its high resistance to peptic hydrolysis was isolated from beef skeletal muscle. It represents at least 2% of the total proteins and is different from known fractions of muscle. Its physiologic role is unknown. The term *peptomyosin* is proposed for this substance. A similar fraction has been extracted from horse skeletal muscle.  
H.J.P.

#### CONCENTRATED AND DRY MILK; BY-PRODUCTS

164. Report on sampling and analysis of condensed buttermilk. RAGNAR E. BERGMAN, State Dept. of Agr., St. Paul, Minn. J. Assoc. Offic. Agr. Chemists, 30, 4: 613. 1947.

The total solids content of a sample of condensed buttermilk was determined by drying at 100° C. under vacuum, drying at 100° C. without vacuum, drying at 70° C. with vacuum, drying at 70° C. without vacuum, and by the toluene distillation method. The average results obtained by 10 collaborators using the above methods were 27.71, 27.21, 29.59, 29.26, and 29.98%, respectively. When the same methods were used, but with 2 g. of recently ignited zinc oxide and 5 ml. of water added, the results obtained by the first 4 methods were 28.67, 28.04, 29.19 and 29.22%, respectively; the toluene distillation method was not used with this procedure. The use of zinc oxide tended to prevent charring of samples. It was recommended that collaborative work on sampling and analysis of condensed buttermilk be continued.  
F.J.B.

#### DISEASES

165. The clinical diagnosis and treatment of breeding unsoundness in cows. J. W. CUNKELMAN. J. Am. Vet. Med. Assoc., 112, 852: 292-295. April, 1948.

Failure in conception is not always the fault of the female. A large percentage of diagnosed conception failures can be treated successfully.  
T.M.L.

#### FEEDS AND FEEDING

166. The value of supplementary vitamins for calves raised under artificial conditions. A. A. SPIELMAN, D. B. H. DALRYMPLE, C. L. NOR-



TON, J. K. LOOSLI, AND K. L. TURK. *Am J. Vet. Research*, 9, 30: 26-29. Jan., 1948.

Results of a large-scale field trial to determine the value of supplementary vitamins for calves raised under good, fair and poor farm conditions of feeding and management are reported. In the first trial, alternate calves received daily, from birth to 30 days of age, a tablet of 5,000 I.U. of vitamin A, 250 mg. vitamin C, 1,000 USP units vitamin D, and 100 mg. niacin. The other calves received a placebo capsule. In experiment 2 the dosages of vitamins were increased. The feeding of extra vitamins to newborn calves, raised under farm conditions of good, fair and poor feeding and management, did not materially reduce the incidence or duration of scours or result in any apparent improvement in the general appearance and condition of the calves up to 30 days of age. T.M.L.

167. Vitamin A and trace minerals in the diet of dairy cattle. P. E. NEWMAN. *Cornell Vet.*, 28, 1: 69-78. Jan., 1948.

The need for adequate vitamin A in the diets of dairy animals becomes more apparent each year. One common source of vitamin A deficiency is in young calves which have not received enough milk for necessary needs; the other is in cows which are pregnant and need an extra amount of vitamins above that found in the average ration.

Cobalt deficiency can be easily corrected by feeding cobalt sulfate in salt at a rate of 1 oz. to 100 lb. Over-feeding of this mineral is dangerous. No general regions for iron deficiency have been observed. Copper is deficient in Florida. Several Great Lakes States and a few others are deficient in iodine. Although manganese is needed by dairy cattle, no clear-cut deficiency areas have been observed. Present information does not indicate a likelihood of a natural deficiency of boron, zinc or fluorine. Caution should be exercised in the supplementing of trace minerals due to the lack of information on exact needs. T.M.L.

### ICE CREAM

168. Basic ice cream industry stabilizers. C. D. DAHLE AND W. F. COLLINS, Pa. State College, State College. *Ice Cream Field*, 50, 6: 24, 25, 26, 35. Dec., 1947.

With a mix containing 12% fat, 11% serum solids and 15% sugar, a perfect texture score was obtained with ice cream having 0.5% gelatin 150 Bloom, 0.42% gelatin 200 Bloom, and 0.35% gelatin 250 Bloom. The pH of gelatin did not affect the results. Dariloid (sodium alginate) was found to give a perfect texture in the above mix when 0.275% was used. Sodium alginate from another source gave comparable results when used in concentration of 0.15%. Locust bean gum (carob) is not satisfactory



as a sole stabilizer because of excessive wheying off of the mix; 0.25% is required for stabilization. Locust bean gum works very well for ices and sherbets and is most important for hot pack cream cheese.

Dried extract of Irish Moss commonly is used to prevent sedimentation in chocolate milk drinks. As little as 0.08% will provide for good mix stabilization but will give high initial viscosities and contribute to a permanent foam. It commonly is used in mixed proprietary stabilizers. Sodium carboxy methyl cellulose, commonly known as CMC or cellulose gum, is tasteless and odorless. When used to the extent of 0.15 to 0.18% in ice cream mixes, it gives good stabilization and results in low viscosity mixes. Slight separation may occur in the mix after storage for about 2 weeks at 35 to 40° F. Pectin commonly is used in ices and sherbets. It was found that 0.15 to 0.25% of 150 grade pectin in ice cream did not properly stabilize the product, although no separation occurred in the mix, which is contrary to previous claims. Ground psyllium seed husks used to the extent of 0.15% gave ice cream with perfect body and texture. Unless a coarse mesh strainer was used ahead of the homogenizer, a considerable amount of the stabilizer was removed. This stabilizer gives a low viscosity mix. Karaya gum long has been used in ices and sherbets and has been employed in mixed stabilizers. About 0.35% is required to stabilize an ice cream mix, but it was found that the smooth texture was of short duration.

The authors report that all of the stabilizers gave satisfactory overrun and that there were not objections to melt down characteristics if the proper amounts were used. In dipping trials with ice creams stabilized with gelatin, Dariloid, CMC and dried extract of Irish Moss, there was no difference in the amount of ice cream dipped from a 2.5-gallon can when the ice cream was tempered to 6° F.

W.C.C.

169. Outlook for cream and milk solids in 1948. J. M. PUNDERSON, Rochester Dairy Coop., Rochester, Minn. *Ice Cream Rev.*, 31, 6: 52, 71. Jan., 1948.

Plenty of butterfat and milk solids for the manufacture of ice cream will be available in 1948, but the price for these products is not expected to drop as low as it did in 1947. Strong domestic consumer demand for fluid milk and cream, export demand, low carry-over stocks of dairy products, and unfavorable milk production trends are cited as the reasons for believing that neither butterfat nor milk solids will show any significant price decline during 1948.

W.J.C.

170. Ice cream mix. A. LEVITON. (Dedicated to the people of the U.S.) U. S. Patent 2,433,850, Jan. 6, 1948 (4 claims). *Official Gaz. U. S. Pat. Office*, 606, 1: 74. 1948.

It is claimed that in an ice cream which is subject to sandiness, the

addition of 3.5 mg. of riboflavin per 100 g. of water will cause the lactose to crystallize in thin trapezoidal plates, a form which does not produce the suggestion of sandiness in the mouth. Other dairy products, such as sweetened condensed milk, also may be made free of lactose graininess through the addition of riboflavin.

R.W.

171. Controlling labor costs in retail outlets. ANONYMOUS. *Ice Cream Rev.*, 31, 6: 42-44, 110. Jan., 1948.

A practical procedure which has been used successfully by one company to meet the challenge of increasing labor costs in the operation of retail stores is presented. Determination of sales by hours is accomplished by hourly checks of the cash register for a week every month for each store with an operating staff of 5 or more persons. The per cent of gross sales can be allowed for labor cost and thus the total amount of money which can be spent daily for labor is determined. This in turn may be figured on the basis of sales per man hour as a labor efficiency guide.

The next step is to determine how the daily man hours should be distributed by hours of the day to provide adequate help for all phases of store operation. Once this has been established the next job is to work out the labor shifts so the proper number of employees will be present when needed. Help for part-time shifts usually can be provided by ladies in the neighborhood who desire part-time employment.

The final step is to prepare a master list of shifts which describes each shift in terms of duties and numbers of hours of daily work. The master list of employee shifts provides a convenient simple method for telling old and new employees what days they work, the hours they are on duty and when they will have their days off.

The charts accompanying this article should prove valuable to any manager of a retail store who wishes to analyze the labor setup for his particular store or group of stores. It is reported that one firm operating 50 fountains in San Francisco was able to reduce store operating hours from two shifts to one in approximately 50% of its locations because of excessive labor costs during lean hours of the day.

W.J.C.

172. Billboard advertising. ANONYMOUS. *Ice Cream Rev.*, 31, 6: 45. Jan., 1948.

Members of the International Association of Ice Cream Manufacturers now may obtain at cost from their association billboard posters for use in an outdoor advertising program to supplement their regular advertising activities. These posters are attractive in design and feature top-notch promotional ideas to stimulate ice cream sales. A place for the imprint of the individual firm is provided so the billboard becomes an effective promotional activity of the firm making use of this service.

W.J.C.

## MILK

173. Program for improving milk quality. E. M. BARKER, Rochester Dairies, Rochester, Minn. Milk Dealer, 37, 4: 102-112. Jan., 1948.

The following broad fundamental or basic principles are listed as essential in a successful quality improvement program: (a) The management of a particular dairy enterprise must be sold on the merits of an improvement program and willing to allocate a substantial sum of money each year over a period of years for its promotion. This naturally involves the development and continued utilization of markets which will pay for quality products. (b) Through such a program economic benefits must accrue over the years to the participating producers. (c) The establishment of differentials and incentives is necessary, based on grades which will accomplish the specific objectives desired. Milk quality will improve more rapidly and will be maintained at a desired level. (d) The recognition that mere regulation will not perform the rightful task of the particular institution and producer is essential. Both management and producers must not fail to recognize that together they must develop and maintain a satisfactory program.

In addition, all producers must use the methods and equipment necessary to continuously supply high quality milk. The milk from such producers must be uniformly and regularly graded according to rigid, prescribed standards. All information pertaining to the milk supply of the producer must be passed on as rapidly as possible to him. A field force, properly directed and made up of well-trained, practical-minded men, is necessary. Platform testing under the supervision of trained intake men is a continuous task. Cans and can washers must be maintained in a satisfactory and sanitary condition. A constant program of education and service to producers must be in effect at all times.

C.J.B.

174. More economical system of homogenizing milk. J. V. QUIGLEY, Chapman Dairy Co., Kansas City, Mo., AND W. A. CORDES, Sealtest, Inc., New York, N. Y. Milk Dealer, 37, 4: 41, 42, 116-124. Jan., 1948.

A new process of homogenizing milk, in which milk is separated and only the cream homogenized, is revealed. The cream going to the homogenizer has a fat content of 8 to 9%. Trials have indicated that satisfactory results will be obtained when the test of the cream does not exceed 13.0%. This process, used with a short-time, high-temperature pasteurizing unit in a closed system, has been in successful commercial operation since Feb., 1945. It has reduced the time of operation of the plant on homogenized milk to about one-third of the time required for the homo-



genization of whole milk, thus resulting in economies involving power, steam, light, refrigeration and labor. Homogenized milk produced by the new process has been demonstrated to be a satisfactory product as judged by top and bottom test differentials, curd tension, microscopic appearance and sedimentation due to leucocytes. C.J.B.

175. Delivery problems relating to the single service container. D. DEAN, Dean's Dairy, Champaign, Ill. Milk Dealer, 37, 4: 46, 100. Jan., 1948.

The delivery problems relating to the single service container are divided into the two distinct categories of long distance hauling and local wholesale and retail delivery. The company has found that the larger units of the tractor trailer type are the most practical for long distance hauling. Smaller units, or so-called "straight jobs" designed to carry 5 tons or less, are too small for a long haul, resulting in overloading of the motor, tires, etc., and the possibility of running afoul of the existing local and state highway regulations. Smaller trucks also are economically unsound because the pay load is not large enough to cover the cost of a union driver whose wages are governed by a union scale covering the larger inter-state trucking companies. In local delivery the type of truck required is essentially the same as that used in delivering milk in glass bottles. It is possible to haul a much greater pay load on a local wholesale or retail truck when single service containers are used. Elaborate plans for refrigeration are not necessary, although ice may be used as a precaution in extremely hot weather. Trucks should be insulated as much as possible, with partitions back of the driver. C.J.B.

176. How to lose money in the milk business. C. F. ROSEBRUGH. Can. Dairy Ice Cream J., 27, 1: 34-42. Jan., 1948.

The tangible ways to lose money in the milk business are through processing and bottled goods shrinkages, ticket and bottle discrepancies, and credit losses. The intangible losses include price cutting, waste plant capacity, waste vehicle capacity, waste manpower capacity, lack of organization, lack of accounting information, and lack of uniform industry costs. H.P.

177. Milk can production in Austria. ING. OTTO WOLFRUM, Vienna, Austria. Milk Dealer, 37, 4: 74-80. Jan., 1948.

The milk cans produced in Austria are described in full and comparisons made with those produced in the United States. Photographs are used for illustration. C.J.B.

178. Portable milk pasteurizing apparatus. E. F. MANGOLD. (One half assigned to H. P. Chapman.) U. S. Patent 2,436,585, Feb. 24, 1948 (8 claims). Official Gaz. U. S. Pat. Office, 607, 4: 704. 1948.

An ordinary 10-gallon can of milk is placed on a platform in a portable container. A pump operated by a motor circulates water from the bottom of the container through a heater and sprays it around the neck of the milk can. The same motor operates a small propeller which keeps the milk agitated.

R.W.

179. Fiberboard cream separating milk container. C. E. DEARDORFF. (Assigned to C. E. Deardorff, Inc.) U. S. Patent 2,436,140, Feb. 17, 1948 (6 claims). Official Gaz. U. S. Pat. Office, 607, 3: 494. 1948.

A paper milk bottle is described which contains a horizontal partition at about the place where the cream line forms. As the milk creams in the container, the cream collects in the upper compartment, from which it may be removed by pouring. During the pouring process the skim milk is retained in the lower section as the result of the V-shaped edge of one side of the dividing partition.

R.W.

180. Separator. K. S. WRISLEY. U. S. Patent 2,436,029, Feb. 17, 1948 (2 claims). Official Gaz. U. S. Pat. Office, 607, 3: 467. 1948.

A V-shaped tubular siphon-type separator for removing cream from bottles of creamed milk has for its chief novel feature a telescopic arrangement which permits adjusting the opening to any desired level within the bottle.

R.W.

181. Milk bottle cap. H. W. BUDAN. U. S. Patent 2,434,787, Jan. 20, 1948 (2 claims). Official Gaz. U. S. Pat. Office, 606, 3: 476. 1948.

A cap or closure for glass milk bottles is described which is designed to be used by the consumer from the time the milk bottle is opened until its contents is finally consumed. It may be made of metal or other material sufficiently stiff, yet elastic, that it will lock itself to the top of the bottle by a skirt or projections provided for the purpose. An integral part of the device is a hook which easily and cleanly removes the customary paper board disc cap used to close glass milk bottles.

R.W.





## ABSTRACTS OF LITERATURE

### BOOK REVIEW

182. Industry builder. The biography of Chester Earl Gray. ROBERT E. JONES. 233 pp. Pacific Books, P. O. Box 558, Palo Alto, Calif.

This work is a history of the Dry Milk Institute as well as an interesting biography of the life of Chester Earl Gray, giving his family background and portraying the American way of life. In it are listed most of the names of men who have contributed toward the progress of the dairy industry, particularly in the dry milk branch, during the past generation. Anyone interested in the progress of the dairy industry should have a copy of this work.

R.B.S.

### BACTERIOLOGY

183. A study of variability in duplicate standard plate counts as applied to milk. J. L. COURTNEY, Milk and Water Laboratory, Oak Ridge Department of Health, Oak Ridge, Tenn. Milk Plant Monthly, 36, 12: 22-24, 27-28, 30. 1947.

A standard plate count may be duplicated by the same laboratory with an average variation of less than 20%. Some variations are surprisingly small. Milk samples were plated experimentally by each of 4 methods. The human error often extends the actual variation in plate counts greatly beyond their proper proportions, while the result seemingly is the normal variation of the method employed. Consciousness of the abuse of the standard plating procedure and a definite effort to improve the technic employed will make the standard plate count a more valuable tool in control programs.

G.M.T.

184. Improved techniques for the microscopic analysis of milk. G. H. WATROUS, JR., Pa. State Coll. Milk Plant Monthly, 36, 10: 42-43. 1947.

Details are given for the strip-counting technique for the microscopic analysis of milk, as well as for suggested stain and staining techniques. The authors find that less variability between operators and less fatigue on the part of technicians examining large numbers of samples result when prescribed procedures are used.

G.M.T.

185. Psychrophiles, mesophiles, thermophiles, and thermodurics—what are we talking about? J. C. OLSON, JR., Univ. of Minn. Milk Plant Monthly, 36, 11: 32-36. 1947.

Mesophilic, thermophilic, thermoduric and psychrophilic bacteria are terms used for moderate heat-loving, heat-loving, heat-enduring and cold-loving groups of bacteria, respectively. There are no well-defined temperature zones for each of these classes of bacteria. Each group may present a special problem and many require a different method of control. Sixteen references are cited. G.M.T.

186. The significance of certain bacteria in pasteurized milk. M. L. SPECK, N. C. State Coll., Raleigh, N. C. Milk Plant Monthly, 37, 2: 36-38, 43. 1948.

Bacteria which have significance regarding sanitary practices employed during the processing of pasteurized milk are, mainly, thermoduric micrococci, thermophilic bacteria, coliform bacteria, and microbacteria. The occurrence of any group in large numbers in milk should be interpreted as a warning that more serious trouble in the form of milk-borne diseases or loss of consumers from the sale of unpalatable milk may result if immediate steps are not taken to correct the faulty practices which permit them to be present. G.M.T.

187. The action of penicillin in-vitro on organisms found in bovine mastitis. H. F. FARRAG. J. Am. Vet. Med. Assoc., 112, 854: 371-374. May, 1948.

A number of organisms commonly found in milk were incubated with cultures containing various concentrations of penicillin. Micrococci were found to be highly sensitive to penicillin action and were fairly uniform in susceptibility. Different strains of *Streptococcus agalactiae* varied significantly in susceptibility to penicillin, while strains of *Staphylococcus aureus*, isolated from the same source, were much more uniform in sensitivity. In one case of streptococcic mastitis milk which was heavily contaminated with *Escherichia coli*, 50% of the penicillin was destroyed when incubated with the milk filtrate for 3 hr. It is suggested that the action of the enzyme, penicillinase (thought to be produced by *Escherichia coli*), may explain some of the variable results from treatment with penicillin.

T.M.L.

## BUTTER

188. Butter defects—their causes and prevention. V. J. BRIMBLECOMBE. Australian J. Dairy Technol., 3, 1: 36-39. 1948.

The causes and remedies of a large number of defects are given in

brief form in this resume of an address. Flavor and aroma defects, body and texture defects and color defects are included. F.E.N.

## CHEESE

189. Making Gouda cheese in Queensland. W. J. PARK. Australian J. Dairy Technol., 3, 1: 34-36. 1948.

In this abstract of an address, quite complete directions for the manufacture of Gouda cheese are given and the causes of defects and the means of prevention also are discussed. Of particular interest is the procedure for salting the curd before hooping. A report of the discussion of the address is included. F.E.N.

## CHEMISTRY

190. On the Babcock test for fat in dairy products. L. R. SCHARP, D. I. SHEW, G. LOFTUS HILLS, R. TREMBATH, AND H. R. WEBB. Australian J. Dairy Technol., 3, 1: 15-23. 1948.

This is a report of a sub-committee of the Victorian Division of the Society of Dairy Technology. The specifications on apparatus and methods given in British Standards Institute Pub. 755, parts I and II, were preferred to the A.O.A.C. methods. The 8% bottle rather than the 10% one was preferred, with the accuracy of calibration within + 0.05%. The BSI standards for pipette calibration and operation were favored; the top of the meniscus should be used for measuring the milk sample. Specific gravity of the acid should be standardized to + 0.002 instead of the + 0.005 commonly used. Addition of acid in one lot was approved. The A.O.A.C. specifications for tester speed and temperature were preferred. Standardized lighting for reading tests is advocated. The average reading of the Babcock test was considered 0.04% higher than by the Röse-Gottlieb ether extraction method. Reducing the quantity of milk delivered by the pipette to 17.82 g. was the procedure preferred for correcting this difference; reading all milk tests to the nearest 0.1% below the actual reading was the correction of choice until a change in pipette specifications was adopted universally. Brief recommendations for testing cream also are given.

F.E.N.

191. Configuration of vaccenic acid. P. C. RAO AND B. F. DAUBERT. Dept. of Chem., Univ. of Pittsburgh. J. Am. Chem. Soc., 70, 3: 1102-1104. March, 1948.

The infrared pattern of vaccenic acid (isolated from beef tallow according to Bertram) was compared with oleic acid and elaidic acid; the *trans* configuration of vaccenic acid is confirmed. H.J.P.



192. Studies on lactoglobulins. J. A. BAIN AND H. F. DEUTSCH. Dept. of Chem. & Physical Chem., Univ. of Wisconsin. Arch. Biochem., 16, 2: 221-229. Feb., 1948.

Fractionation procedures of both bovine and goat lactoglobulins by means of alcohol precipitation methods (Cohn) are described. Lactoglobulins showing one peak upon electrophoresis at a given pH were obtained. More than one component, however, was revealed when pH mobility curves were determined. On sedimentation analysis, both preparations showed one component, but only the bovine lactoglobulin appeared to be molecularly homogeneous. H.J.P.

193. The thermodynamics of metallo-protein combinations. Copper with bovine serum albumin. I. M. KLOTZ AND H. G. CURME. Chem. Lab., Northwestern Univ., Evanston, Ill. J. Am. Chem. Soc., 70, 3: 939-943. March, 1948.

The extent of binding of cupric ions by bovine serum albumin was measured by the equilibrium dialysis technique at pH 4.8 and 0 and 25° C. Free energies, entropies and enthalpies have been calculated for the multiple equilibria involved. The cation-protein linkage is through the carboxyl group; a stable complex can be formed when the carboxyl group is in suitable juxtaposition with other substituents or residues. H.J.P.

194. The binding of some sulfonamides by bovine serum albumin. I. M. KLOTZ AND F. M. WALKER, Chemical Laboratory, Northwestern Univ., Evanston, Ill. J. Am. Chem. Soc., 70, 3: 943-946. March, 1948.

The formation of complexes between 6 sulfonamides and crystallized bovine serum albumin was investigated. The energy of binding data has been correlated with structural features of the drugs. H.J.P.

195. Ionic exchangers in the dairy industry. O. F. GARRETT, M and R Dietic Laboratories, Inc., Columbus, Ohio. Milk Dealer, 37, 6: 50, 132-140. March, 1948.

The ionic exchange process is explained by describing the zeolite process of treating hard water. When hard water is passed through a bed of zeolite, the calcium is removed from the water and held by the zeolite; at the same time sodium is given up by the zeolite to the water, thereby converting the scale-forming calcium salts to non-scale-forming sodium salts. The total mineral content of the water remains virtually unchanged. The end result is soft water. The cationic and the anionic types of exchangers are described.

The following applications of this new basic process to dairy products are outlined: The preparation of soft curd milk; the preparation of soluble sodium caseinate; the treatment of wheys, whereby essentially 100% of the lactose present in the original whey is recovered; the preparation of dried cream products containing from 50 to 70% butterfat which reconstitute well in water, are perfectly stable in hot coffee, and do not curdle when used with acid fruits or in making creamed soups; and the stabilization of evaporated milk.

C.J.B.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

196. Milk and milk products in bread making. F. R. DAVIDSON. Australian J. Dairy Technol., 3, 1: 40-42. 1948.

The addition of lard or a similar fat at the time ordinary skim milk powder is added to the dough mix or the use of a milk powder containing the fat which was emulsified into the milk before drying is reported to overcome the volume defects associated with the use of 6% milk powder in bread. This amount of powder is considered necessary to increase properly the nutritive value of bread. In addition to the resume of the address, a report of the discussion which followed also is given.

F.E.N.

197. Aeration of butterfat containing liquids. C. A. GETZ. (Assigned to Aeration Processes, Inc.) U. S. Patent 2,435,682, Feb. 10, 1948 (6 claims). Official Gaz. U. S. Pat. Office, 607, 2: 280. 1948.

Whipped cream is produced by aerating the cream in a variety of ways with nitrous oxide gas, with or without such other gases as cyclopropane, dimethyl oxide, methyl chloride or difluorodichloromethane. It is claimed these gases permit the use of cream containing 30% fat or lower, the use of homogenized cream and unaged cream. The whipped cream exhibits no clumping of fat globules, the serum drainage is minimized and bacterial growth inhibited.

R.W.

198. Factors to consider in making high quality chocolate milk drinks. W. C. THACKER, Farmers' Cooperative Dairy, Winston-Salem, N. C. Milk Plant Monthly, 36, 1: 30-32. 1948.

Six desirable characteristics of chocolate milk are: (a) Use of high quality milk or part-skimmed milk, (b) mild chocolate flavor, (c) little or no sedimentation accompanied by low viscosity, (d) elimination of ragged, off-colored cream layer, (e) light to medium red color, and (f) medium to high sweetness. The amount of chocolate used in chocolate milk ranges from 1 to 1.5% of the finished product. When liquid chocolate is used, the amount ranges from 1.50 to 2.25%. Sedimentation is minimized by eliminating in-



soluble particles, increasing fineness of grinding, increasing the viscosity of the product, and decreasing the difference in density between the suspended particle and the suspending medium. Homogenization does not prevent settling of cocoa in chocolate milk. Stabilizers vary in their ability to prevent sedimentation. Creaming can be prevented by pasteurizing the milk at 160–165° F. for 15–30 min. A 5% sugar is suggested, while an 8% sugar is about the maximum sweetness tolerated. Production abuses often arise when processors do not follow the specific directions accompanying chocolate syrups. G.M.T.

199. Evaluation of procedures for the manufacture of cottage cheese. W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, 36, 11: 28–30, 70, 72. 1948.

Much of the cottage cheese today is made from pasteurized skim milk. The previous heat treatment given the skim milk may determine the amount of heat required to cook the curds. Among the known factors which affect the amount of heat required to cook cottage cheese are the acidity of the curd and whey, the concentration of the curd-forming constituents, the size of the curds, the amount of agitation, the types of acids present and the presence of gas. Some commercial practices involve gas formation, which heretofore has been discriminated against. Under certain prescribed conditions gassy curds may be cooked economically and the curds firmed rather quickly. G.M.T.

200. The manufacture of cottage cheese. A. A. SCHOCK. S. Dak. State Coll. Milk Plant Monthly, 36, 10: 82–86. 1947.

The manufacture of cottage cheese involves: (a) pasteurizing the milk at 143° F. for 30 min.; (b) setting the milk at 86° F. by adding 5 lb. of starter for 100 lb. of milk and the addition of 1 ml. of rennet per 1,000 lb. of skim milk, plus 2 oz. of 50% calcium chloride per 1,000 lb. of skim milk if formation of desirable coagulum shows this addition necessary; (c) cutting the curd at a whey acidity between 0.45 and 0.55%; (d) cooking the curd slowly to a temperature yielding a desired firmness of curd; (e) draining and washing the curd with cold water within a 0.5-hr. period; and (f) creaming so that the fat content is approximately 4% and salting at the rate of 1 to 1.5 lb. per 100 lb. of cottage cheese. The defects in cottage cheese manufacture are slow setting, tough and rubbery curd, and soft and pasty curd. Remedies are suggested for these defects. G.M.T.

201. Preparation of kefir fermented milk. L. A. BURKEY, Bureau of Dairying, U. S. Dept. of Agr. Milk Plant Monthly, 37, 1: 48–49. 1948.

Kefir fermented milks are acid milks which have been fermented by



means of kefir grains. These may be obtained in dry form from the American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C. The kefir grains may be perpetuated by transferring them every 2 or 3 days into a new supply of skim or whole milk by rinsing them in clean cold running water before transfer. The kefir milk may be prepared by heating the milk to 165° F., cooling to 70° F., suspending the kefir grains at the rate of 1 part to 3 parts of milk and incubating at 70° F. Large quantities of kefir buttermilk may be obtained by suspending the kefir grains in cheese-cloth bags at the surface of the milk. When sufficient acidity (about 1%) has been developed, the grains may be removed and the milk agitated to produce uniform smoothness, then cooled and bottled. G.M.T.

202. The manufacture of cultured buttermilk. A. A. SCHOCK, S. Dak. State Coll. Milk Plant Monthly, 36, 10: 48-50, 52, 54. 1947.

See Abs. 120, J. Dairy Sci., 31, 4: A46. April, 1948.

#### FOOD VALUE OF DAIRY PRODUCTS

203. Studies on the comparative nutritive value of fats. X. On the reputed growth-promoting activity of vaccenic acid. H. J. DEUEL, JR., S. M. GREENBERG, EVELYN E. STRAUB, DOROTHY JUE, C. M. GOODING, AND C. F. BROWN. Dept. of Biochemistry and Nutrition, Univ. of Southern Calif., Los Angeles, and Bayonne Laboratory, The Best Foods, Inc. J. Nutrition, 36, 3: 301-314. March, 1948.

When the diet of rats contained either cottonseed oil or butterfat, no difference in rate of growth was found. When the diet contained rape-seed oil, growth was less. Decreased efficiency of utilization of the rape-seed oil was attributed to its high content of eracic acid. Vaccenic acid or hydrogenated China wood oil fed to the rats on the rape-seed oil diet did not increase growth, nor did vaccenic acid fed to rats receiving the cottonseed oil diet. R.K.W.

#### ICE CREAM

204. Evaluation of vanilla. J. MERORY. Ice Cream Rev., 31, 8: 54, 56, 60, 62. March, 1948.

Manufacturers and chemical users of vanilla flavor must learn to use their senses of smell and taste in the evaluation of vanilla. Results of laboratory analysis are of value in protecting the consumer against fraud, but they do not in themselves indicate the quality of vanilla flavor. Basic factors which determine the quality of vanilla flavor include quality of vanilla bean used, care given the bean before and after extraction, method

of extraction, quality of menstrum, procedure for blending and aging of the vanilla extracts, and type of container used during aging.

Vanilla flavor is obtained by extraction only, and not more than a 4-fold concentration of vanilla is possible by extraction. Distillation is to be avoided, since vanilla flavor is difficult to evaporate and the process tends to destroy the fragrance of the aromatic compounds of the vanilla beans.

Manufacturers of vanilla extracts should insist that in the purchase of vanilla beans the complete nomenclature of origin, grade, size, date of cure, and moisture content be shown on the label of the container in which they are packed. The chemical reaction which produces vanillin and other aromatic compounds in the bean continues as long as the moisture content remains unchanged. Storage of beans, therefore, under suitable conditions for a prolonged period of time prior to extraction is deemed advisable.

Typical analytical values are given for vanillin, total ash, lead number, alkalinity of total ash, soluble ash, color and resin of vanilla extracts, and applications of these values are discussed.

W.J.C.

205. Average butterfat content in ice cream 12% during 1947. ANONYMOUS. *Ice Cream Trade J.*, 44, 3: 52. March, 1948.

The average butterfat content of ice cream in 1947 was 12% compared to 10.3-10.6 during 1944 and 1945, when government controls were in effect. This figure is considerably above the minimum butterfat requirement in many states. Five states have an 8% minimum, 23 states a 10% minimum, 14 states a 12% minimum, 1 state a 13% minimum, 4 states a 14% minimum, and one state has no standard.

W.H.M.

206. High temperature-short time pasteurization of ice cream mix. C. M. MINTHORN, Chester Dairy Supply Co., Chester, Pa. *Milk Plant Monthly*, 37, 2: 70-73. 1948. Also *Ice Cream Rev.*, 31, 8: 45, 97, 98, 100. 1948

Ice cream mix may be pasteurized successfully by high temperature-short time pasteurization. Usually a higher temperature than the standard 160° F. for 15 seconds is employed. The temperature recommended is 176° F. for 22 seconds. The author concludes: (a) Ice cream mix can be heated without burn-on continuously for long periods of time. (b) Heating of ice cream mix containing frozen fat from storage temperature to 125° F. in less than 1 min. prevents oxidation of the fat. (c) High temperature-short time pasteurization decreases the amount of equipment, with a saving of time and labor. (d) When mix is prepared in a condensing pan, high preheating temperatures can be used for increased efficiency of the condensing equipment and, even more important, for stabilizing milk solids.

G.M.T.



207. **Controlling processing operations.** J. C. NESMITH, Southern Dairies, Inc. *Ice Cream Rev.*, 31, 7: 54, 56. Feb., 1948.

In the control of labor costs in the ice cream plant, 4 factors of major importance are discussed. (a) New employees should be advised in writing of all company rules and policies so there will be no chance for misunderstandings developing at a later date. It is suggested that the company in turn should keep a permanent and complete file on each employee. (b) A weekly work schedule setting forth the kind and amount of each product to be manufactured should be planned in advance. In addition, a daily work schedule should be prepared which will show the hours each employee is to be on duty and what the nature of his duties will be. (c) Overtime pay should be eliminated. To accomplish this, have employees dress for work before punching the time clock; allow a staggered 15-min. rest period each morning and afternoon; have the timekeeper check all pay cards a day or two before the close of each week's operation to determine if time is in line with the normal work week—if not, an adjustment in work schedule may be necessary. (d) Records should be kept so that the actual labor cost on each and every product may be determined. The detailed information for such records can best be obtained by the department foremen who, in turn, will give the information over to the accounting department. The accounting department will then be able to supply the management with accurate labor-cost figures on each item manufactured. W.J.C.

208. **Full utilization of the plant laboratory.** H. McAULIFFE, Bowman Dairy Co., Chicago, Ill. *Ice Cream Trade J.*, 44, 3: 56, 89-93. March, 1948.

Most ice cream manufacturers recognize the need for a laboratory but fail to utilize one properly. Considerable time can be saved by deciding what information is desired and necessary. Decisions on how samples should be taken, who should take them and how they should be cared for are just as important as deciding what samples should be taken.

The effectiveness of the laboratory often is lost because of lack of authority or failure to exercise its authority to follow through and take steps to prevent the recurrence of faulty practices. The laboratory should be used not only to check dairy ingredients, but its activities should be expanded to include examination of non-dairy ingredients and, in some instances, paints, fuels and other materials purchased. Developmental research on modification of the product can be handled with the control program. The laboratory can be used to give some technical training to new employees. It also can be used as a part of a public relations program. W.H.M.

209. **Shrinkage.** H. A. BENDIXEN, State College of Washington, Pullman. *Ice Cream Trade J.*, 44, 2: 46, 47, 90-94. Feb., 1948.

The following precautions are suggested for guarding against shrinkage:



(a) Avoid freezing the ice cream too stiff in the continuous freezer, since under such conditions the air in the ice cream might be present under considerable pressure. (b) Avoid extreme temperature changes or heat shocking of the ice cream, especially if a high overrun is obtained. If ice cream is hardened with dry ice or in very low-temperature freezing tunnels, do not change the ice cream suddenly to high temperatures. A more gradual change is preferable. (c) Use high-quality, low-acid dairy products to prevent destabilization of the proteins. (d) Avoid an excessively high sugar content and especially a high dextrose content, which would increase the fluidity of the ice cream at any given temperature of storage. (e) Avoid the use of unparaffined cartons or cans, banging of the packages, and excessive air circulation directly over the ice cream in the storage room.

W.H.M.

210. Present status of ice cream shrinkage. J. A. LEEDER, Ramsey Laboratories, Cleveland, Ohio. *Ice Cream Rev.*, 31, 8: 152. March, 1948.

Suitable temperature control of ice cream during freezing, hardening and storage is regarded as the most important factor involved in the shrinkage problem. The following recommendations are made with respect to temperature control as a means of guarding against shrinkage: Avoid freezing ice cream excessively stiff and dry at the freezer. Maintain ice cream hardening rooms at  $-10$  to  $-20^{\circ}$  F., with distributing branches at  $-5^{\circ}$  F. Avoid temperatures below  $-20^{\circ}$  F. Maintain uniform temperatures in all hardening and storage rooms. Do not load trucks with insufficiently hardened ice cream. Transport trucks should be maintained at below  $0^{\circ}$  F. and retail trucks at  $-2$  to  $+4^{\circ}$  F. Avoid exposure of ice cream to dry ice in trucks or in cabinets. Maintain retail cabinets at  $-15^{\circ}$  F. or below.

Other factors mentioned which may cause shrinkage or produce ice cream susceptible to shrinkage are: Use of sweetened condensed milks; use of excessive amounts of dextrose or other sweeteners which contain a high mineral content or which are high in acidity; use of stabilizers containing an emulsifier such as polyhydric alcohol ester or the use of egg yolk; high overrun; incorporation of air pockets into cans or containers as they are filled; use of paper containers which are not properly paraffined on the inside surface.

W.J.C.

211. The retail store, past—present—future. Part 2. D. GHORMLEY. *Ice Cream Trade J.*, 44, 3: 46-48, 93-96. March, 1948.

The development of the retail store from 1935 to 1942 is discussed. Expenses, merchandising, standardization of products and location are among the factors important for successful operation.

W.H.M.

212. Sherbets, the status of. ANONYMOUS. *Ice Cream Trade J.*, 44, 3: 50, 101-102. March, 1948.

The production of 12,520,000 gallons of sherbet in 1947 was down 18% from 1946 and 81% below the record 1945 production according to statistics issued by the U.S.D.A. The volume of sherbets made in 1947 was still 50% greater than that of the last pre-war year, 1941. It appears that sherbets have returned to the minor status that they occupied during the pre-war years in the over-all industry picture.

W.H.M.

213. The new cabinet look. ANONYMOUS. *Ice Cream Field*, 51, 1: 30, 32, 34. Jan., 1948.

The merchandising program of Sealtest, National Dairy Products Corp., New York, built around the "Ice Cream Mart" is described. The mart consists of an 8-hole ice cream cabinet, enhanced by a front of baked enamel and a counter of stainless steel. Provision is made for display of attractive advertising material. Ten points are listed upon which the merchandising program is based. Ice cream is an impulse-buying item; the mart is for the exclusive sale of ice cream; strict cleanliness should be observed in the use of the mart. Since April, 1947, about 4,000 sales marts have been installed by Sealtest dealers in principal towns and cities of 33 states.

W.C.C.

214. The efficient use of cabinet and truck space. L. C. ANDERSON, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 44, 1: 42-43, 68-70. Jan., 1948.

The problem of how much cabinet space to furnish, how many deliveries to make, and the size of the trucks which need be used is one that cannot be made the subject of some mathematical formulae but must be studied from the standpoint of individual markets and individual customers within such markets. A study should be made to secure data as to the proper size of cabinet to furnish a customer, the proper number of days to make deliveries and the day of the week on which to make deliveries. Such a study would make it possible to establish a sound cabinet and delivery policy and eliminate excessive cabinet holes and excessive number of deliveries. The determination of the proper delivery days will assure the dealer ample stock and maximum sales.

W.H.M.

215. Stimulating volume through maximum use of cabinets. W. D. DOBSON, Carnation Co., Los Angeles, Calif. *Ice Cream Trade J.*, 44, 3: 54-55, 99-101. March, 1948.

Information on how to build sales volume through better use of cabinets,

reducing costs through optimum use of equipment, and the cost of furnishing cabinets and cabinet rental schedules is presented. In dry stops, the cabinet should be where it can be seen and where there is heavy store traffic; near the check stand usually is a good location. A lighted super structure, a tilted mirror showing the inside of the cabinet, and attractively arranged stock are points which have been used effectively by chain stores. In wet stops, visual display of packaged and bulk ice cream has been found to increase sales. The display of related items such as cones, cakes, cookies and toppings may be used to attract attention and sell these items, as well as ice cream, in increased amounts.

The right-sized cabinet is needed for the various accounts; studies should be made at frequent intervals to determine if the cabinets are being utilized effectively. Cost figures should be determined on cabinets of various sizes. For example, this company has determined that a 12-hole, double-row conventional cabinet costs \$10.34 per month, consisting of: depreciation (6 yr.), \$3.94; interest (6% on the unamortized balance), \$1.06; field labor servicing cabinets, \$1.06; maintenance and material, \$0.31; shop repair and labor, \$1.62; and overhead in cabinet department, \$2.35. W.H.M.

216. A study of ice cream delivery practices. ANONYMOUS. Ice Cream Trade J., 44, 2: 44, 45, 85. Feb., 1948.

Information is presented on frequency of delivery, driver salesmen's wages, commissions, cabinet investments, size of cabinets, and size of new equipment. Thirty-two per cent of the replies from manufacturers indicated 3 deliveries per week in summer and 2 in winter was most efficient. Various other combinations, up to daily delivery, were suggested. Location of store with respect to the plant or on a route was listed most often as influencing the number of deliveries which should be made. There was no uniformity in replies regarding the frequency of delivery to accounts of different size. However, 47% thought that 5 gallons was the least amount that could be delivered economically and a majority thought that 100 gallons per year was the least amount of sales that justified an investment in a cabinet. In reply to the question regarding the preference for larger cabinets and trucks, 68% of the manufacturers stated that they would buy larger ones. Forty-five per cent of the manufacturers pay drivers a salary and commission. Commissions range from 1 to 6 cents per gallon.

W.H.M.

217. A study of ice cream delivery practices. ANONYMOUS. Ice Cream Trade J., 44, 1: 38-40, 73-74. Jan., 1948.

The high points of a survey of 1945 delivery practices of 350 companies made by the Statistical and Accounting Department of the International



Association are as follows: (a) The cost of distribution constituted a smaller share of the operation in 1945 as compared with 1941, dropping from 29.05% of the expense dollar in 1941 to 20.19% in 1945. Distribution cost includes delivery and customer service and selling. (b) Cost of products used in ice cream increased from 45.43% of the expense dollar in 1941 to 54.6% in 1945—an increase of more than 20% in cost, which was offset to a large extent by the drop in distribution cost. (c) Manufacturing and administrative costs in the 2 years were about the same. Administrative cost in 1941 was 5.57% of the expense dollar and 5.76% in 1945; manufacturing costs were 19.95% and 19.37%, respectively. (d) More than four-fifths of the ice cream volume (87.09%) is handled via the peddle system in delivering ice cream. (e) Most cabinets from which ice cream is sold are loaned to dealers with no rental charge; 61% of the cabinets were in this category. However, a surprising number of cabinets (18.6%) were rented to dealers and 20.4 were owned by the dealers. (f) The manufacturers who use 2 of the foregoing methods of cabinet service sell 53.88% of the ice cream; 16.79% use all 3 methods; 20.19% use only 1 method; and 9.14% did not answer. (g) The frequency of delivery was limited by 87.12% of the ice cream manufacturers, and this group sold 90.99% of the ice cream. (h) Of those answering the survey, about 47.5% felt that 5 gallons was the least amount that could be delivered economically; 26.02% thought 10 gallons; 6.85%, 15 gallons; and 4.11%, 20 gallons. However, 5.93% felt that they could deliver as little as 2.5 gallons economically. (i) Some 45.71% of the ice cream manufacturers pay a salary and commission to their driver salesmen; 5.4% pay a commission only. The most popular commission was 1 cent per gallon, with 2 cents per gallon the next most popular method.

W.H.M.

**218. "Dry stop" merchandising equipment makes its debut. ANONYMOUS.**

Ice Cream Trade J., 44, 1: 46-47, 70-73. Jan., 1948.

"Dry stop" merchandising equipment, with its display and advertising facilities, not only enables the "dry stop" dealer to direct attention to his ice cream products, but also gives him an opportunity to promote the sale of factory-filled packages on a greater scale than ever before and provides him with the means for selling cones, sundaes, frappes, hot fudge, carry-out bulk and other items not requiring carbonation. A detailed description of suitable units is given.

W.H.M.

**219. Package production for consumer acceptance. J. C. PFEFFER, G. P.**

Gundlack & Co., Cincinnati, Ohio. Ice Cream Trade J., 44, 2: 52, 54, 83-85. Feb., 1948.

Sanitation, package design, and mix composition are among the factors that will influence favorably the sale of packaged ice cream. There seems

to be a demand for both the regular 12% and the deluxe high-fat, low-overrun ice cream.

The standard package is one of about 12% fat and 39% total solids, with about 75 to 80% overrun and frozen in a continuous freezer and hardened quickly in 6 to 7 hr. The purpose of the deluxe package is to make a product which will be accepted by the consumer who has demanded bulk dipped ice cream.

W.H.M.

220. 1947 gallonage. ANONYMOUS. *Ice Cream Trade J.*, 44, 2: 48, 95, 96. Feb., 1948.

The 1947 production of ice cream was 622,400,000 compared to 708,913,000 in 1946, or a 12% drop, according to the preliminary report of the U.S.D.A. Bureau of Agricultural Economics.

W.H.M.

221. Price trends. V. M. RABUFFO. *Ice Cream Trade J.*, 44, 1: 34-35, 64-68. Jan., 1948.

The sky-rocketing cost of ice cream ingredients, together with other increases, has resulted in an increase of 8 to 15 cents per gallon for bulk and packaged ice cream in most of the larger cities. Typical selling prices for bulk vanilla ice cream are as follows: New York City, \$1.66; Up-state New York, \$1.60 to \$1.65; Boston, \$1.60 to \$1.65; Chicago, \$1.76; Los Angeles, \$1.45; Milwaukee, \$1.55; Minneapolis, \$1.15; South, \$1.50; Philadelphia, \$1.52; Pittsburgh, \$1.47; and Cincinnati, \$1.45. Pint packages are priced in New York at \$1.86—up 16 cents per gallon, and the higher-fat French type was up 24 cents per gallon, to \$2.24. Retail prices generally have advanced from 2 to 5 cents on factory-filled pints.

W.H.M.

## MILK

222. Off-flavors in market milk. A. A. SCHOCK AND D. F. BREAZEALE, S. Dak. State Coll. *Milk Plant Monthly*, 36, 9: 28-30, 32, 48-49. 1947.

The authors summarize the causes of off-flavors of milk. The milk off-flavors are classified as follows: (a) those transmitted by the cow, (b) those absorbed directly by the milk from the atmosphere, (c) those that gain entrance into milk directly from bacterial contamination, and (d) those resulting from enzymatic, chemical and photochemical changes occurring in the milk. In order to provide consumers with wholesome milk having a pleasing flavor, milk processors should pay especial attention to intake selection of milk, cleanliness of equipment, stainless steel construction, processing temperatures, and a daily check on the bottled product. Special emphasis is placed on the importance of having a well trained man who knows milk flavors at the receiving platform.

G.M.T.

223. Determination of time taken in three phases in a H.T.S.T. pasteurizer. J. V. PASCOE. Australian J. Dairy Technol., 3, 1: 3-5. 1948.

A. 0.1% water solution of methylene blue was introduced into the pipe from milk pump to regenerator by dissembling when the machine, running on water, was stopped. Upon resumption of pumping, 3-oz. samples were taken at 3-second intervals entering the holder from the heating plates, entering the regenerator after holding and leaving the regenerator before entering the brine cooler. Dye concentrations in the samples were determined with a Zeiss Pulfrich Photometer, after necessary dilution. The average holding time was 37.5 seconds, but some material came through in 18 seconds.

F.E.N.

224. A laboratory high-temperature short-time pasteurizer. J. V. PASCOE. Australian J. Dairy Technol., 3, 1: 5-7. 1948.

An apparatus based upon rapid and controlled changes in temperature of the heating and cooling water surrounding aluminum tubes containing 1.5 ml. of milk agitated by aluminum plungers within the tubes is described. The apparatus has been used to duplicate heating, holding and cooling times of commercial pasteurization equipment.

F.E.N.

225. Cream bodying. G. G. GIBSON, Sidney Wanzer and Sons, Inc., Chicago, Ill. Milk Dealer, 37, 5: 43, 44, 100. Feb., 1948.

Methods by which the body or viscosity of cream can be increased are reviewed and discussed. A practical method based on 12 yr. of experience is outlined. Separate the cream as near the desired test as possible but always be sure that the fat is high enough for the desired test. It is much easier and better on the cream body to bring the test down by adding milk or skim than it is to use heavy cream to bring the test up. Cool immediately to 50° F. or less. Standardize and pasteurize. Cool to 40° F. or lower. Allow to stand for at least 0.5 hr., preferably 2 hr. or longer, if possible. Warm cream to 82-87° F., depending upon such things as season of year, type of vat, speed of agitation, and cream used. Hold 5 min., without agitation, after reaching desired temperature. Cool cream to 42° F. and watch cream closely from this point on in cooling; as soon as an increase in viscosity appears, stop agitation. If no increase occurs, cool cream to 38° F. Allow cream to stand at cooled temperature for at least 0.5 hr. or longer (up to 2 hr.) if possible. Just before bottling, agitate cream until desired viscosity is reached. This may take only 10 sec. or it may take 10 min., but at this point the cream can be put near the same viscosity every day. Check viscosity of the cream after it is bottled and again after 24 hr. to determine what kind of viscosity cream has when the consumer receives it.

C.J.B.



226. Ten common causes of excessive sediment in milk. Part 8. Milking machine suction cups. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. *Milk Plant Monthly*, 36, 9: 70-72. 1947.

The use of a milking machine does not necessarily insure freedom of extraneous matter in the milk, as indicated by farm sediment checks. Sediment found in milk indicates 3 principal sources of contamination, namely, dirt on the teats; suction cups dropping down during the milking process, where they pick up shavings, bedding and other extraneous matter; and from careless storing of the suction cups themselves. G.M.T.

227. Three-day milk delivery. ANONYMOUS. *Milk Dealer*, 37, 6: 40, 102-104. March, 1948

Three-day-a-week milk delivery in Columbus, Ohio, is no longer an experiment. Thoroughly tested on a marketwide basis since it was first introduced in Oct., 1943, 3-day delivery is enthusiastically endorsed by Columbus milk dealers, routemen, plant employees, and consumers. Milk is received from the farm 7 days a week. Sunday milk-receiving requires a skeleton plant crew on that day—enough men to operate the receiving room and storage department. All other plant men and all drivers have Sundays off.

The production advantages and disadvantages and delivery advantages and disadvantages are given. The problem of inadequate storage space in refrigerators of consumers and in plant storage rooms was partially solved through the adoption of the square bottle. C.J.B.

228. Billing retail customers. E. THOM. *Milk Dealer*, 37, 5: 40-42, 132-136. Feb., 1948.

Sending out monthly statements to retail milk route customers can and does range from a highly complex accounting and bookkeeping system, complete in every detail, to a method over-simplified to a point where it consists merely of the routeman leaving a note stating "You owe \$9.20". A general picture of some of the forms now used in the industry and some of the advantages and disadvantages encountered by the users of these forms is presented. The information is based on a questionnaire sent out to a selected list of milk dealers, both large and small, in all parts of the country. C.J.B.

## SANITATION AND CLEANSING

229. A review of the literature pertaining to the chemistry of can washing. L. L. LITTLE, E. F. Drew & Co., Inc., Boonton, N. J. *Milk Plant Monthly*, 36, 11: 22-24, 26, 38, 40-41. 1947.

Washing compounds for cans should have properties of detergency, in-

hibition of film and scale formation, and prevention of corrosion. Film formation can be prevented through the use of sequestering agents. Corrosion rate of metals increases rapidly with decreasing pH, there being no justification for the general belief that organic acids are inherently less corrosive than inorganic acids when compared on a uniform pH basis. The detergent properties of the various alkalis have been firmly established through many years of application in cleaning operations. Detergents comprising alkalis, condensed phosphates, and synthetic detergents may be expected to act on the soil through physical action, base exchange, electrochemical action, saponification, and surface activity. Acid cleaners may be expected to act on soils through dissolving milkstone, physical action, and surface activity. Forty-six references are cited. G.M.T.

230. Planned dairy plant sanitation. M. P. BAKER, Dept. of Dairy Industry, Iowa State College, Ames. Milk Dealer, 37, 6: 118-122. March, 1948.

The sanitation of dairy plants is discussed under inspection of equipment, operations, store rooms and other non-processing rooms, and premises. The article is summarized as follows: Plant sanitation is related to efficiency of operation, quality of products and to public relations. In a planned program of plant sanitation it is important to outline first the objectives, the procedures to be followed and the time of the activities connected with the program. The management should be actively interested and, whenever possible, take an active part. Records should be kept in detail; they help in measuring progress and also in organizing desirable changes as their need becomes apparent. C.J.B.

231. Once upon a time there was a neglected milking machine. J. KEENAN, Pennsylvania Salt Manufacturing Co. Milk Dealer, 37, 6: 42, 43, 94. March, 1948.

Good milk utensil care is summarized. Before milking, rinse all utensils with water containing 200 p.p.m. chlorine. Use same strength, hot, for cleaning cows' udders. Immediately after milking, rinse all utensils with clean cold water. Then brush all utensils with hot water containing a good soapless dairy washing powder, and suck it through the milker units. Hang cup units of milking machine on rack and fill with fresh lye solution. Use 4 level teaspoons of lye to each gallon of water. Drain after 20 min. in freezing weather. Dismantle milker at least once a week, clean thoroughly, and replace worn or faulty rubber. Twice a month, give rubber the hot lye soak treatment, using 1 heaping tablespoon of lye to each gallon of water. Scrub all parts after rinsing with a good milk stone remover solution. C.J.B.

## MISCELLANEOUS

232. Excessive "oiling off" of frozen cream can be prevented. G. M. Trout, Michigan State College. Food Freezing, 2, 9: 628, 629, 641, 648. Aug., 1947.

The results of research of a number of workers, including that of the author, on the control of oiling-off of frozen cream are presented. Addition of 10 to 15% sugar prior to freezing, while the best practical control procedure, places limitations on the use of the cream. Quick freezing, rapid defrosting, separation of milk when the fat is in the solid state, and immediate freezing without holding the precooled cream, slightly improve the stability of the fat emulsion. Homogenization, effective in re-emulsifying oiled-off cream, does not stabilize the fat emulsion when such cream is frozen and thawed.

L.M.D.

233. A quality control program for dairy plants. J. H. Health, Southern Dairies, Winston-Salem, N. C. Milk Plant Monthly, 37, 2: 57-59, 69. 1948.

A quality control program for dairy plants should include field, plant, and laboratory control. Field control should work toward the production of more high quality milk, convincing the producer that the plant is sincerely encouraging him to produce economically high quality milk. Plant control might well be summarized as good housekeeping. Laboratory control involves certain qualitative checks on quality of the milk, tests for butterfat, bacteria, phosphatase, acidity, coliform, bottle sterility, and homogenization efficiency.

G.M.T.

234. How employer-employee relationships reflect good management. J. W. Post, Armour and Co., Chicago, Ill. Milk Dealer, 37, 6: 76-82. March, 1948.

Nothing in business that affects profit and loss has changed so radically in the past 7 or 8 yr. as have the people who perform the details of the operation. The following favorable assets of a good employee are given and briefly discussed: Careful workmanship, experienced and interested in present work, industrious, rapid worker, exercises initiative and resourcefulness, versatile, cooperative with supervisors, cooperative with associates, economical, good housekeeper, makes friends, health, punctual, and practices safety rules.

The following functions of management are given: (a) To analyze, to determine what is to be done, how it is to be done, and when it is to be done. (b) To train representatives to perform properly the what, how and when of the operation. (c) To direct those operations as changes in outside influences (such as competition, markets, legislation and emergencies) require. The Taft-Hartley Act and employer development also are discussed.

C.J.B.



235. Water treatment in the dairying industry. A. K. BEENIE. Australian J. Dairy Technol., 3, 1: 24-32. 1948.

Water softening by various methods, demineralization, clarification, cooling systems in which water is used, boiler water treatment, water for washing purposes and bacteriological treatments are discussed in relatively general terms.

F.E.N.

236. The preparation of peaches for freezing. Part IV. J. G. WOODROOF, ETHEL SHELOR, S. R. CECIL, AND IDA ATKINSON. Food Freezing, 2, 9: 632-634. Aug., 1947.

This excerpt from the Georgia Experiment Station Bulletin 251 gives details of prevention of browning of peaches. Ascorbic acid and citric acid are used in combination, and 4 formulas, 2 for home use and 2 for commercial freezers, are given. These formulas are recommended as the result of 2 yr. of experimentation with several dozen packs of several varieties of peaches. High conversion corn sirup was found to be fully as effective as 50% cane sirup for sweetening peaches, its use being limited to 50% of the total sugar solids in a pack. A brief discussion of packaging is given, bringing out the needs of containers for the various consumer usages. L.M.D.

237. Brine freezing strawberries in tin. C. F. ELLIS AND J. B. WEGENER, Food Processing Research Station, TVA. Food Freezing, 3, 1: 22, 56. Dec., 1947.

A report is made of immersion freezing of 1:4 pack sliced strawberries in no. 10 cans in a 34% chromated calcium chloride brine, circulated at approximately  $-27^{\circ}\text{F}$ . At the end of 60 min., the unfrozen core was 3 in. in diameter, 4.5 in. deep, and had a frozen circumference 1.5 in. in depth. The temperature of the unfrozen core had dropped to 42 from the initial temperature of  $73^{\circ}\text{F}$ . There was no definite freezing point, but solidification apparently was completed within the range of 22 to  $24^{\circ}\text{F}$ .

On the basis of these findings, a commercial freezer put into operation a can immersion freezer of 5,850 lb. per hr. capacity using 0 to  $-10^{\circ}\text{F}$ . brine. Following 54 min. of immersion freezing, the cans of berries were washed free of brine in a water spray, put in cartons, and placed in a zero storage room which had adequate coil surface for the completion of freezing. Samples taken from time to time showed that at the end of 54 min. the unfrozen core temperature was  $44^{\circ}\text{F}$ . Approximately 60% of the heat removal necessary for freezing had been done by the time the cans were removed from the freezer and the temperature of the unfrozen contents had been lowered sufficiently to arrest fermentation. This method of operation had several commendable features: (a) a brine temperature of  $-10$  to  $0^{\circ}\text{F}$ . allowed moderate suction pressures at the compressors; (b) after the first batch, there was continuous operation; (c) the major portion of heat ex-

traction load was handled in the freezer; (d) the capacity of storage rooms greatly increased without the danger of an excessive temperature rise; and (e) faster freezing resulted in rapid temperature drop through fermentation range compared to doing all freezing in the storage room even if designed for a heavy load. L.M.D.

238. The truck-trailer industry moves to keep abreast frozen food progress. J. B. HULSE, Truck-Trailer Mfrs. Assn., Washington, D. C. Food Freezing, 3, 1: 36-37, 56. Nov., 1947.

This is the first half of an article clarifying misconceptions existing as to the facilities available for truck transportation of frozen foods and setting forth progress that has been made in the design of low temperature truck-trailer bodies and their refrigeration to handle frozen food transfer safely and efficiently. The author emphasizes that the refrigerated trailer vehicle is not intended to be used as a food processing plant. The advantages and disadvantages of the existing refrigerating systems are given. L.M.D.

239. Food Freezing's survey of state locker laws and regulations. ANONYMOUS. Food Freezing, 2, 10: 693-695, 710, 712. Sept., 1947.

The rapid assumption of status as a public service agent is evidenced in the wide scope of state locker plant laws and regulations enacted since the adoption of the first such statute by Iowa in 1939. Today 22 states have specific locker laws, and all but a handful of the others provide control through adaptations of existing statutes to cover regulatory action by state departments. The main portion of the article embodies a tabular condensation of locker plant laws for the 48 states, showing a striking lack of uniformity and inadequacy of protection of the patron against poor practices. On the other hand, there is evidence of a growing tendency toward more implicit adherence to the technological advances in the food-freezing field. L.M.D.

240. Steps in the handling of frozen fish in the freezer warehouse. J. M. LEMON, Technological Sec., Division of Commercial Fisheries, Fish and Wildlife Service. Food Freezing, 2, 9: 606-608, 642-645. Aug., 1947.

A trial was made of flavor absorption of butter stored at 10° F. with packaged fish. The butter, ordinarily wrapped and 93 score, was placed in sealed tins also containing various species of fish fillets individually wrapped in cellulose-base moisture-vapor-proof material. One control sample was held by itself. In 337 days the control without fish scored 91, and 2 samples with sole and oysters scored 91.5, while 1 sample with salmon scored 89. Proper protection should prevent any odor absorption by butter. L.M.D.

